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1. Authors should discuss briefly the quality control methods used to ensure that the data are complete and accurate. A reliable procedure should be cited for ensuring that all patients entered on study are actually reported upon. If no such procedures are in place, their absence should be noted. Any procedures employed to ensure that assessment of major endpoints is reliable should be mentioned (eg, second-party review of responses) or their absence noted.

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3. The study should not have an inevaluability rate for major endpoints of greater than 15%. Not more than 15% of eligible patients should be lost to follow-up or considered inevaluable for response due to early death, protocol violation, missing information, etc.

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7. All claims of therapeutic efficacy should be based upon explicit comparison with a specific control group, except in special circumstances where each patient is his own control. If nonrandomized controls are used, the characteristics of the patients should be presented in detail and compared to those of the experimental group. Potential sources of bias should be adequately discussed. Comparison of survival between responders and nonresponders does not establish efficacy and should not generally be included. Reports of phase II trials which draw conclusions about antitumor activity but not therapeutic efficacy generally do not require a control group.

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9. The methods of statistical analysis should be described in detail sufficient that a knowledgeable reader could reproduce the analysis if the data were available.

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* *From* Methodologic Guidelines for Reports of Clinical Trials (Cancer Treat Rep 69:1–3, 1985).

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Trilostane Therapy for Advanced Breast Cancer[1]

R. C. Coombes,* T. J. Powles, J. Muindi, J. Hunt, M. Ward, D. Perez, and A. M. Neville[2,3]

Forty-one postmenopausal patients with advanced breast cancer have been treated with trilostane, a 3βD-hydrogenase Δ^5-isomerase inhibitor, for periods of up to 1 year. One patient responded to trilostane and in six patients the disease was stabilized. The remaining patients failed to respond to therapy. Six patients who failed to respond to trilostane subsequently responded to other forms of endocrine therapy. It is concluded that trilostane alone is not a useful agent in the treatment of advanced breast cancer. [Cancer Treat Rep 69:351–354, 1985]

Trilostane (fig 1) is a synthetic compound which inhibits the 3β-hydroxy-steroid dehydrogenase-Δ^5-3 oxosteroid isomerase enzyme system (1). The action is competitive and reversible and its sites of action are shown in figure 2. Aminoglutethimide, another steroid synthesis inhibitor, acts in two different sites in the steroid synthetic pathway, namely, at the desmolase and the aromatase enzyme system. Initial studies in patients with Cushing's syndrome have shown that trilostane is effective in reducing cortisol in this syndrome (2). Other studies have indicated that trilostane also reduces estrogen levels and may be of value, when combined with steroids, in causing regression of human breast cancer (3). We therefore carried out preliminary studies to assess the value of trilostane, without steroid replacement, in patients with advanced breast cancer.

PATIENTS AND METHODS

Patients

Forty-one patients with advanced breast cancer and measurable metastatic disease were treated with trilostane. All patients were fully assessed by conventional staging methods including photography and clinical examination, biochemical screen, bone and liver scanning, and chest and skeletal radiography, as detailed previously (4), prior to and at 3 and 6 months after the start of therapy. Thereafter, patients were staged at 6-month intervals. Assessment was by strict International Union Against Cancer criteria as previously described (5).

All patients were postmenopausal, but six had undergone surgical castration. The age range was 47–84 years, with a mean of 63.8 years.

All patients had had prior endocrine therapy as follows: eight had received tamoxifen alone; three had received aminoglutethimide alone; 12 had received a combination of tamoxifen, aminoglutethimide, and danazol; two had received danazol alone; two had had oophorectomy only; and 13 had had multiple forms of endocrine therapy. A total of 21 patients had received aminoglutethimide either alone or in combination. No patient had received endocrine therapy or chemotherapy within 4 weeks of starting trilostane, and all patients had progressive disease.

Trilostane Dose

The first ten patients who received trilostane were treated with an escalating dose from 120 to 900 mg/day over a period of 6 weeks. Blood pressure, electrolytes, and cortisol were measured and toxicity was assessed using a questionnaire.

In the remaining 31 patients therapy was started at a dose of 240 mg and escalated every 2 weeks unless untoward side effects occurred. The final doses achieved in the patients were as follows: unable to re-

[1]Received May 18, 1983; revised Sept 11, 1984; accepted Sept 12, 1984.

[2]Ludwig Institute for Cancer Research (*R. C. Coombes, J. Hunt*, and *A. M. Neville*), and Department of Medicine (*R. C. Coombes, T. J. Powles, J. Muindi, M. Ward*, and *D. Perez*), The Royal Marsden Hospital, Sutton, Surrey, England.

[3]We thank Drs. D. Segal and M. McDonald from Sterling Winthrop for supplying Modrenal (trilostane) and the Biochemistry Department, The Royal Marsden Hospital, for the measurements of electrolytes and cortisol. We are indebted to the Nursing Staff on Smithers Ward (The Royal Marsden Hospital) for their help in this study. We also thank other clinicians from The Royal Marsden Hospital who allowed us to study their patients.

Reprint requests to: Dr. R. C. Coombes, The Royal Marsden Hospital, Downs Rd, Sutton, Surrey, SM2 5PT, England.

ceive therapy due to side effects (one patient), < 240 mg (four), 240–480 mg (25), and > 480 mg (ten). Only one patient received 960 mg of trilostane.

Estrogen receptor (ER) analysis was available in 21 of 41 patients. Measurement was by a modification of the method of McGuire et al (6). Sixteen patients' tumors were ER-positive, ie, containing > 10 fmols of ER/mg of cytosol protein, and five were negative, containing < 10 fmols/mg of cytosol protein.

RESULTS

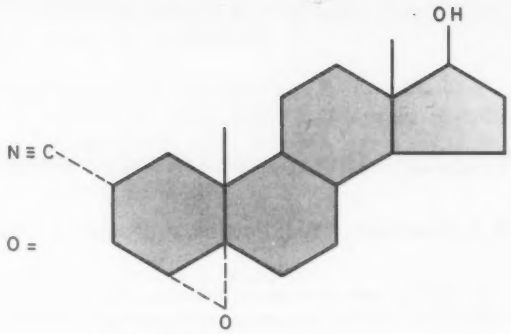
Effect of Escalating Dosage in the First Ten Patients

Trilostane had no effect on systolic or diastolic blood pressure or on measurable cortisol, sodium, or urea (fig 3). Two patients showed a consistent rise in serum potassium but only within the normal range. Because of cross-reaction of the drug in radioimmunoassays for estrogen, androstenedione, and testosterone, no other measurements of hormones were obtainable.

Response in Patients

Forty-one patients had therapy lasting from 7 days to 1 year. Seven patients had treatment for < 3 weeks, eight patients stopped therapy between 3 and 8 weeks, and 25 patients were treated for 8–52 weeks. One patient was lost to follow-up soon after starting therapy.

In one patient, some soft tissue skin nodules regressed for 4 months; six of 40 patients (15%) showed stabilization of their disease from 3 to 12 months. Four of these patients were relieved of bone pain, and one patient's pleural effusion regressed. The remaining patients' disease progressed. Sites of disease in treated patients (ie, bone, visceral, and soft tissue) are shown in table 1. If only patients are considered who received therapy for ⩾ 8 weeks, then seven of 25 patients (28%) showed stabilization or response.



TRILOSTANE
(4α, 5-epoxy-17β-hydroxy-3-oxo-5α-androstane-2α carbonitrile)

FIGURE 1.—Structure of trilostane.

The single responder and four of six patients with stabilization of disease had all responded to prior endocrine therapy. Thirteen of 33 patients with progressive disease also responded to prior endocrine therapy and six of these patients went on to respond to other endocrine agents (aminoglutethimide, three patients; tamoxifen, one; and danazol, two) after trilostane therapy was discontinued. Five of the patients showing stabilization of disease and the single responder had ER-positive tumors.

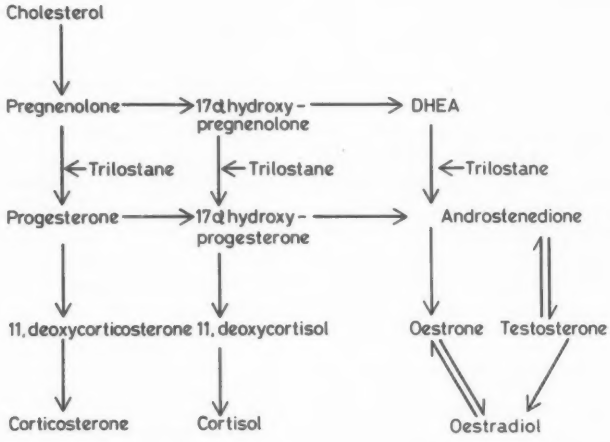


FIGURE 2.—Sites of action of trilostane.

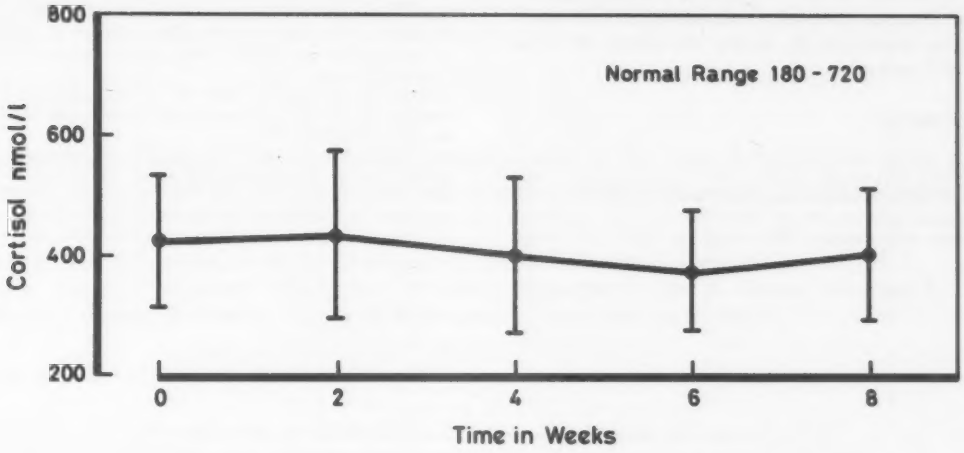


FIGURE 3.—Effect of trilostane on serum cortisol levels (mean ± SD) of 9 patients.

Effects Observed and Their Relationship to Dose

Fourteen patients received < 480 mg of trilostane daily. In six patients, this was due to rapid progression of disease as the dose was being escalated. In six patients, side effects prevented the patient from receiving further therapy. One patient was too elderly and one was lost to follow-up.

Seventeen patients received 480–540 mg of trilostane daily; in 13 the dose was not increased because of side effects. One patient required surgery and no reason was given for not escalating the dose further in three patients.

There was no relationship between the dose of trilostane and the likelihood of response or stabilization. Thus, two of 14 patients receiving < 480 mg/day had stable disease, whereas four of 17 patients receiving 480–540 mg/day had stable disease or responded. Only one of ten patients who received 720 or 960 mg/day responded.

Side Effects of Therapy

The most common side effects were lethargy (ten patients) and nausea (ten patients). Two patients experienced severe diarrhea and two experienced dyspepsia. Two patients became hypercalcemic and an additional two had hypotension and giddiness, both of which were reversible by steroids administered. A single patient had palpitations which reversed on stopping therapy. Twenty-three of 40 patients had no side effects.

DISCUSSION

Trilostane alone does not appear to be effective against metastatic breast cancer since only a single patient responded to treatment. Some patients who failed to respond to trilostane therapy subsequently responded to other types of endocrine treatment, reinforcing this impression. However, six patients also showed stabilization of disease and appeared to benefit from therapy. In particular, one patient whose pleural effusion diminished after the start of therapy had good symptomatic relief of dyspnea.

These results are particularly disappointing since a similar group of patients had been treated with trilostane and corticosteroid supplements and significant regression of breast cancer was seen with this combination in 26% of patients (3). It may be that addition of corticosteroids is necessary to prevent the reflex rise in adrenocorticotropic hormone that may be engendered by trilostane therapy. This may also permit a higher

TABLE 1.—Sites of disease in patient population*

Response category	Total	Site of disease		
		Bone	Visceral	Soft tissue
Partial	1	0	0	1
Stable disease	6	5	5	5
Progressive disease	33	18	11	23

* Values = No. of patients.

dose of trilostane to be administered, but in this case it would be important to assess the effect of corticosteroids used alone.

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Randomized Clinical Trial of Cyclophosphamide, 5-FU, and Prednisone With or Without Tamoxifen in Postmenopausal Women With Advanced Breast Cancer[1,2]

James E. Krook,[3] James N. Ingle,[4] Stephanie J. Green,[4] Warren D. Bowman,[5] Lloyd K. Everson,[6] Harold E. Windschitl,[7] Robert F. Marschke,[8] John A. Laurie,[9] Stephen A. Cullinan,[10] Delano M. Pfeifle,[11] Greg W. McCormack,[6] and Thomas E. Elliot[3,12,*]

For The North Central Cancer Treatment Group

The purpose of this study was to determine if tamoxifen added to the efficacy of a CFP (cyclophosphamide, 5-FU, and prednisone) regimen. One hundred thirty-one postmenopausal women with advanced breast cancer without prior chemotherapy exposure were randomized to receive CFP alone or combined with tamoxifen; six were disqualified because of ineligibility. Objective responses were seen in 68% of the eligible patients receiving CFP and in 61% of those receiving CFP plus tamoxifen. Median times to progression were 287 days for CFP and 158 days for CFP plus tamoxifen (two-sided log-rank test of equality of progression distributions, $P = 0.07$). Median survival times were 544 days for CFP and 394 days for CFP plus tamoxifen (two-sided log-rank test of equality of survival distributions, $P = 0.08$, after adjustment for stratification factors using the Cox model, two-sided $P = 0.14$). The addition of tamoxifen to CFP was not associated with longer time to progression or survival in any Cox covariate model. On the basis of our data, it can be concluded that the addition of tamoxifen to CFP does not substantially improve either the time to disease progression or survival in women with metastatic breast cancer. [Cancer Treat Rep 69:355–361, 1985]

The concept of combining hormonal therapy with chemotherapy is attractive because the two modalities generally have different mechanisms of action and nonoverlapping toxic effects. Following the introduction of tamoxifen it became clear that this agent possessed activity against breast cancer and was clinically tolerable (1). Combination chemotherapy consisting of cyclophosphamide, 5-FU, and prednisone (CFP) has been utilized in advanced breast cancer, with reproducible objective response rates of 50%–60%, and is judged to be well-tolerated (2). On this basis the North Central Cancer Treatment Group performed the following study to compare the therapeutic efficacy of combination chemotherapy with CFP alone with that of CFP combined with tamoxifen in postmenopausal women with advanced breast cancer.

METHODS

One hundred thirty-one women with histologically confirmed breast cancer and progressive metastatic disease were entered in this study from January 1978 to July 1982. Eligibility requirements included a postmenopausal status defined as > 1 year since last menstrual period or history of prior castration. Women with a prior hysterectomy without oophorectomy must have been > 50 years of age. Patients must not have received any prior cytotoxic chemotherapy or treatment with tamoxi-

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[2]Supported by Public Health Service grant CA-25224 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

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[4]Division of Medical Oncology (*J. N. Ingle*), and Cancer Center Statistics Unit (*S. J. Green*), Mayo Clinic, Rochester, MN.

[5]Billings Clinic, MT.

[6]Fargo Clinic, ND.

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[11]Quain and Ramstad Clinic, Bismarck, ND.

[12]Additional participating institutions include: University of Nebraska, Omaha (*J. F. Foley*); and Iowa Oncology Research Association, Des Moines (*S. F. Brunk*).

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fen. Prior hormonal therapy with other hormonal agents was permissible provided that progressive metastatic disease occurred while receiving that hormone. In patients with a response to prior estrogen therapy, at least 4 weeks must have elapsed from the time of estrogen withdrawal, with progression of disease in the interim prior to entry in this study. Patients were required to have measurable or evaluable disease. A measurable indicator lesion was required to have two measurable perpendicular diameters, except in the case of hepatomegaly, where the liver had to be palpable ≥ 5 cm below a costal margin in the midclavicular line or xiphoid during quiet respiration. A filling defect on liver scan must have measured at least 5 × 5 cm to be considered measurable. Evaluable disease was assessable but not measurable. Patients must have had an Eastern Cooperative Oncology Group (ECOG) performance score (PS) of 3 or better (0, fully active; 4, totally disabled), wbc count > 4100/mm^3, platelet count > 130,000/mm^3, serum creatinine < 2 mg/dl, serum bilirubin < 2 mg/dl, and serum calcium < 12 mg/dl.

Examinations obtained prior to entry in the study included history, physical examination with assessment of indicator lesions, hemoglobin, wbc and platelet counts, chemistry profile, chest radiograph, and metastatic bone survey. An estrogen receptor (ER) analysis was not required for entry in this study, but when available, ER-positive was defined as ≥ 10 fmols/mg of cytosol protein and ER-negative was defined as < 10 fmols/mg of cytosol protein.

Patients were stratified according to ER status, prior hormonal treatment, dominant disease status, ECOG performance status, indicator lesion status, and institution. Patients were then randomized to CFP alone or combined with tamoxifen according to a dynamic allocation scheme (3). The CFP regimen consisted of cyclophosphamide (150 mg/m^2) and 5-FU (300 mg/m^2), each administered iv for 5 consecutive days every 5 weeks, and prednisone, which was given orally at a dose of 30 mg/day for 14 days, then at 20 mg/day for 7 days, and subsequently at 10 mg/day as a maintenance dose. Tamoxifen was given at a dose of 10 mg twice daily.

Patients were assessed every 5 weeks. For both measurable and evaluable disease, a complete response (CR) was defined as the disappearance of all evidence of cancer. For measurable disease, a partial response (PR) was defined as a ≥ 50% reduction in the product of perpendicular diameters of indicator lesions or a greater than or equal to one-third reduction in the sum of linear measurements of the liver below the right and left costal margins in their respective midclavicular lines and xiphoid. Disease progression was defined as a > 25% increase in the product of perpendicular diameters of indicator lesions or the sum of linear liver measurements or the appearance of new lesions. Stable disease was defined as failure to qualify for CR, PR, or disease progression. For evaluable disease, a regression was defined as a definite decrease in tumor size. Stable disease was defined as no definite increase or decrease in tumor size. Disease progression was defined as a definite increase in tumor size or the appearance of new lesions. Time to disease progression was defined as the interval from date of randomization to date of first progression or date of treatment-related death. For patients with inadequate tumor assessments, time to disease progression was censored at the date of last valid assessment. Censorship for disease progression was at time of last assessment for the one patient dying of causes unrelated to disease or treatment.

Chi-square tests and logistic regression (4) were used to analyze discrete data (ie, for response and toxicity). Disease progression and survival distributions were estimated by the Kaplan and Meier method (5). Since sensitivity to proportional hazards alternatives was desired, log-rank tests (6) and proportional hazards modeling (7) were used to analyze times to disease progression and death. Log-hazard ratios and standard errors were estimated from the proportional hazards models and were used to test hypotheses and to calculate confidence intervals on the hazard ratios. Single-stage methods were used throughout, although several interim analyses were performed with intent (not formalized) of stopping early due either to a highly significant difference favoring CFP plus tamoxifen or to results inconsistent with a moderate difference favoring CFP plus tamoxifen. Approximately eight interim analyses would have been anticipated throughout the study, since it was initially projected that 100 patients/arm would be accrued.

PATIENTS

One hundred twenty-five patients were considered evaluable; six patients were disqualified. Four patients were disqualified from the CFP group because of prior chemotherapy (two patients), premenopausal status (one), and incorrect diagnosis of metastatic breast cancer (one). Two patients were disqualified from the CFP plus tamoxifen group because of prior chemotherapy (one patient) and no measurable or evaluable disease (one).

Patient characteristics at the time of entry in this study are shown in table 1. The two groups are relatively well-balanced with respect to ECOG PS, prior hormonal therapy status, ER status, and indicator lesion status. Some imbalances are present with respect to age and dominant disease status.

There is a higher proportion of osseous-dominant patients in the CFP group and a higher proportion of visceral-dominant patients in the CFP plus tamoxifen group. A substantial cause of this imbalance is that in 21% of patients there was an error in designation of dominant disease status at the time of registration of patients, which was due to misunderstandings of definitions during the early phases of this cooperative group

TABLE 1.—Patient characteristics*

Characteristic	CFP	CFP + tamoxifen
Total No. of patients	66	59
Median age in yrs (range)	63 (33-82)	60 (39-80)
ECOG PS		
0,1	74	69
2,3	26	31
Prior hormonal therapy		
Yes, with response	9	8
Yes, no response	26	27
No	65	64
Prior oophorectomy	9	10
ER status		
Positive	29	25
Negative	33	32
Not obtained	38	42
ER status (no intervening systemic therapy)		
Positive	20	17
Negative	30	31
Not obtained	50	53
Dominant disease status		
Soft tissue	15	15
Osseous	30	19
Visceral	55	66
Indicator lesion status		
Measurable	74	75
Evaluable	26	25

* Unless otherwise specified, values = % of patients.

trial. Our randomization procedure maintained balance on the remaining stratification factors at the expense of only slowly correcting the imbalance in dominant disease status. Since the imbalance remaining at the end of the study could affect treatment outcome, patient characteristics were considered in covariate analyses.

In stratifying for ER status, the protocol allowed the use of an ER status from a prior determination if one was not available from a metastatic lesion at the time of entry in the study. Because systemic hormonal therapy intervened in some patients (eight receiving CFP, six receiving CFP + tamoxifen), these data were also examined considering such patients to be in the "not obtained" category. For ER-positive patients, the median ER value was 70 fmols for patients receiving CFP (range, 10-409 fmols) and 106 fmols for those receiving CFP plus tamoxifen (range, 10-438 fmols).

RESULTS

Response Data

One patient who received several cycles of treatment did not have adequate assessment of tumor status to judge response; thus, 124 patients were considered evaluable for response to treatment. The four patients who received only one cycle of therapy and did not return for evaluation due to toxicity were considered to be nonresponders. Table 2 shows response data with a breakdown according to measurable and evaluable disease. The overall response rates (CR + PR + regression) of 68% for CFP and 61% for CFP plus tamoxifen are not significantly different (chi-square, $P = 0.44$). An approximate 95% confidence interval for the difference in response probabilities is from 0.24 to -0.10 (ie, response to CFP is estimated to be between 0.24 higher and 0.10 lower than to CFP + tamoxifen). Thus, it is unlikely that the response probability on CFP is substantially lower than on CFP plus tamoxifen. There was also no significant difference between the measurable or evaluable category (chi-square, $P = 0.30$ and 0.83, respectively). Within the subset of patients known to be ER-positive at the time of entry in this study, the response rates were 77% (ten responses among 13 patients) for CFP and 50% (five re-

TABLE 2.—Response data

Best response achieved	% of patients receiving— CFP	CFP + tamoxifen
No. of measurable patients	49	44
CR	12	14
PR	59	48
Stable disease	24	16
Immediate disease progression	4	18
No return (toxicity)	0	5
CR + PR	71	61
No. of evaluable patients	16	15
CR	19	20
Regression	38	40
Stable disease	31	27
Immediate disease progression	6	7
No return (toxicity)	6	7
CR + regression	56	60
Total responses (CR + PR + regression)	68	61

sponses among ten patients) for CFP plus tamoxifen. These subsets have small numbers of patients and imbalances in patient characteristics, but there is no indication of superiority of CFP plus tamoxifen. Response rates according to dominant disease status for CFP and CFP plus tamoxifen were 80% and 78%, respectively, in patients with soft tissue-dominant disease; 58% and 55%, respectively, in patients with osseous-dominant disease; and 69% and 59%, respectively, in patients with visceral-dominant disease (all differences were not significant). A logistic regression analysis was performed with tumor response as the dependent variable and treatment arm, age (< 65, ≥ 65 years), ER status (positive: yes, no; negative: yes, no), prior hormonal therapy (yes, no), PS (PS 0: yes, no; PS ≥ 2: yes, no), dominant disease status (soft tissue: yes, no; visceral: yes, no), measurable indicator lesion (yes, no), and two institution variables. None of the variables were significantly associated with response.

Survival

Over three-quarters of the patients entered in this study have died. The median time to death was longer for patients treated with CFP (ie, 544 vs 394 days); the two-sided log-rank test of equality of survival curves produced a P value of 0.08 (fig 1). However, the Cox covariate analysis using the variables listed above did not show treatment to be significantly associated with survival. ER-negative status, visceral dominant disease, measurable disease, and PS ≥ 2 were associated with shorter survival (two-sided P values were 0.003, 0.01, 0.04, and 0.06, respectively, in the joint model). Treatment-ER status interaction variables were not significant. Interestingly, improvement due to CFP is estimated to be even greater in ER-positive patients than in ER-negative patients. Treatment was not significant in any model ($P = 0.14$ after adjustment for the significant variables). Results were quite similar using the ER variable with no intervening therapy.

After adjustment for stratification factors, treatment with CFP plus tamoxifen had an estimated proportional effect on the death hazard of 1.38, with a 95% confidence interval of 0.91-2.08. That is, the estimated instantaneous death rate at each point in time of patients receiving CFP plus tamoxifen is 1.38 times that of patients receiving CFP. The hypothesis that CFP plus tamoxifen is 20% better than CFP (ie, instantaneous death rate on CFP is at least 1.20 times that on CFP + tamoxifen) is rejected in favor of the hypothesis that CFP plus tamoxifen is < 20% better, with a single-stage P value of 0.01. It should be noted that due to the multiple tests performed and planned, the true probability of a false conclusion is probably about 0.05 rather than 0.01. The study was terminated because of this inconsistency with even a moderate advantage for CFP plus tamoxifen.

Disease Progression

Of 100 patients, 99 had disease progression and one died of disease while in the study, and three others had possible treatment-related deaths before progression. Ten patients were removed from the protocol due to toxicity and were followed until disease progression on sub-

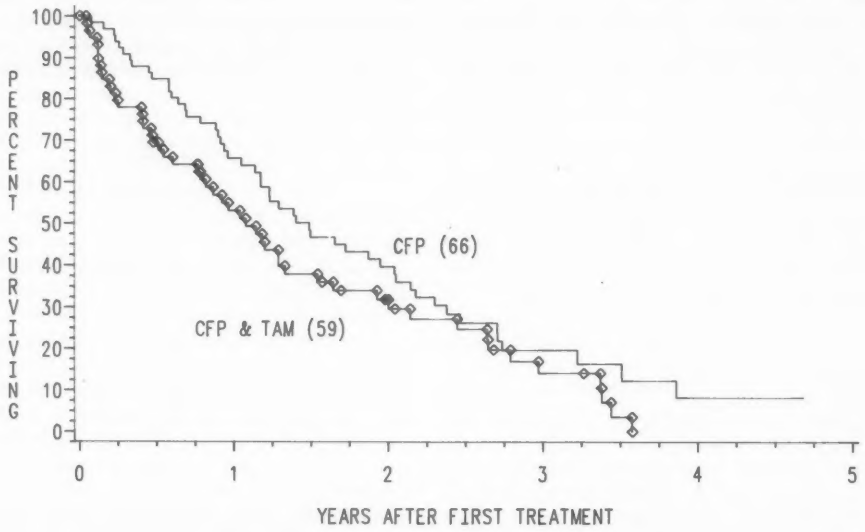

FIGURE 1.—Survival for patients receiving CFP and CFP + tamoxifen.

sequent treatments (four on the CFP arm, three on the CFP + tamoxifen arm) or continue to be followed (one on the CFP arm, two on the CFP + tamoxifen arm). Three patients never had valid tumor assessments and were censored at time on study, thereby decreasing the number evaluable for progression to 122. Of the 122 patients, progression was censored at the time of last valid tumor evaluation for only three patients who refused follow-up. Six patients remain in the study (five on the CFP arm, one on the CFP + tamoxifen arm).

The median time to disease progression was longer for patients treated with CFP than for those treated with CFP plus tamoxifen (ie, 287 vs 158 days, fig 2), but the two-sided log-rank test of equality of distributions did not show a significant difference ($P = 0.07$). Since the proportional hazards assumption may not have been met, a generalized Smirnov test (8) was performed and was also not significant ($P = 0.07$); if adjustment is made for the performance of both tests, the P value is > 0.07 (9).

Cox covariate analysis was performed using the variables listed above. Treatment with CFP plus tamoxifen appears to be worse than treatment with CFP ($P = 0.04$) after adjustment for the significant variables. After adjustment for stratification factors, the estimated progression hazard ratio of CFP plus tamoxifen to CFP was 1.48, with a 95% confidence interval of 1.01-2.18. The results are inconsistent with a 15% improvement for CFP plus tamoxifen over CFP at the 0.01 level (single-stage).

Toxicity (table 3)

Both treatment regimens were judged to be quite tolerable. Hematologic toxicity, defined as a wbc count nadir $\leq 2000/mm^3$ and a platelet count nadir $\leq 130,000/mm^3$, was somewhat greater for CFP plus tamoxifen, but not significantly so. Three patients on each treatment arm had myelosuppressive toxicity which was either a definite or possible cause of death. Nonhematologic toxicity was generally acceptable with relatively small proportions of patients having severe toxic effects.

DISCUSSION

This trial did not show that there was any advantage to the addition of tamoxifen to CFP when used as first-line combination chemotherapy approach in postmenopausal women with advanced breast cancer. If anything, the findings favored CFP alone, suggesting a possible deleterious effect of tamoxifen on this combination chemotherapy regimen. From the standpoint of examining a biological question (ie, an adverse tamoxifen-chemotherapy interaction), it would have been of interest to continue patient accrual to examine a larger number of patients. However, from an ethical standpoint, it was not considered defensible to continue this study to demonstrate that an experimental arm was inferior given these results; patient accrual was terminated prior to reaching initially projected accrual objectives.

This study included patients whose ER status was unknown, but it would be expected that most of them would be ER-positive. In clinical practice, most patients will not have tumors which are both readily accessible and in sufficient quantities for the determination of the receptor status. Ideally, all patients entered in a clinical trial eval-

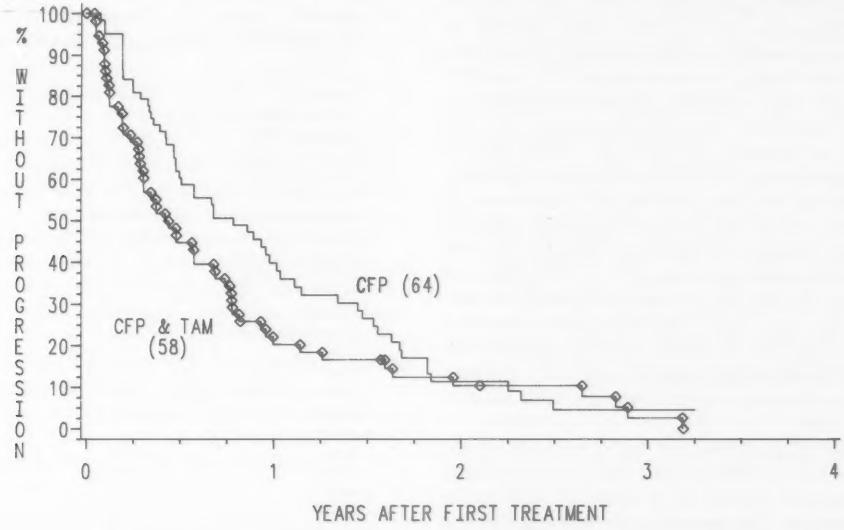

FIGURE 2.—Time to disease progression for patients receiving CFP and CFP + tamoxifen.

uating the impact of hormonal therapy should have the receptor status known. Small sample sizes within known receptor subsets are a problem with our study and limit conclusions which can be drawn with respect to specific subsets. However, we found no indication that the addition of tamoxifen to CFP provided superiority in any ER subset.

Although about two-thirds of our patients had not received prior hormonal therapy, about one-quarter in each group had received prior hormonal therapy without a response as judged by the entering investigator. Inclusion of these patients was considered reasonable in that breast cancer is perceived to be a disease which is heterogeneous with respect to receptor population, and objective regressions have been observed with tamoxifen in patients failing to achieve a response with prior hormonal therapy [eg, an 18% PR rate with tamoxifen in 28 patients failing to achieve a response with diethylstilbestrol (10)]. It may be, however, that inclusion of these patients without a prior response to hormonal therapy, as well as those who were not ER-positive, did introduce confounding factors which could decrease sensitivity to the elucidation of the hormonal therapy/chemotherapy interaction.

Several clinical trials examining the addition of tamoxifen to chemotherapy are of note. Cocconi et al (11) randomized postmenopausal patients without prior chemotherapy exposure to either CMF (cyclophosphamide, methotrexate, and 5-FU) alone or CMF plus tamoxifen. Patients who failed on CMF alone were then given tamoxifen, but the CMF was continued. One hundred thirty-three patients were considered evaluable. For patients receiving CMF plus tamoxifen initially, the response rates were significantly higher (74% vs 51%, $P < 0.01$). When objective remissions were examined according to site of metastases, the statistically significant superiority was limited to bone lesions (69% vs 32%; $P < 0.005$). The time to disease progression was also longer for those treated with CMF plus tamoxifen ($P = 0.06$) (median, 48 vs 24 weeks). However, the overall survival was longer for patients treated initially with CMF alone (median, 111 vs 78 weeks), but this difference was not significant ($P = 0.25$). ER data were available for only 39 (29%) of the patients. Although the response rate was higher for ER-positive patients treated with CMF plus tamoxifen (ie, 12 of 15 [80%] vs six of 13 [46%]), the sample sizes were quite small. The investigators concluded that the chemotherapy/hormonal therapy approach was advisable in situations that urgently required a response but that the sequential approach was advisable for common clinical use.

Tormey et al (12) studied the use of doxorubicin and mitolactol with or without tamoxifen in a randomized trial involving 122 patients, all of whom had received prior chemotherapy. The response rate was significantly higher for the chemotherapy/hormonal therapy approach (55% vs 36%) as was the median time to disease progression ($P = 0.001$; median, 170 vs 110 days). Likewise, the survival was longer for the regimen containing tamoxifen (median, 340 vs 270 days), but this did not reach statistical significance ($P = 0.18$). It was concluded by these investigators that tamoxifen increased the therapeutic effectiveness of the second-line regimen utilized and suggested that the addition of tamoxifen to other chemotherapy regimens would be beneficial. This study by Tormey et al (12) involved a different population of patients than we studied; our patients had not received prior chemotherapy.

Kardinal et al (13) presented an excellent report on 232 evaluable postmenopausal women randomized to either CAF (cyclophosphamide, doxorubicin, and 5-FU) or T-

TABLE 3.—Toxic effects

Toxicity	CFP	CFP + tamoxifen
Hematologic (cells/mm^3)		
Cycle 1		
Leukopenia		
No. of evaluable patients	63	56
Median nadir	4100	3350
Nadir range	1000-12,300	500-8800
% with ≤ 2000	17	29
% with ≤ 1000	5	14
Thrombocytopenia		
No. of evaluable patients	62	56
Median nadir	183,500	162,000
Nadir range	73,000-492,000	25,000-365,000
% with ≤ 130,000	23	36
% with ≤ 75,000	2	5
All cycles		
Leukopenia		
No. of evaluable patients	65	56
Median nadir	1700	1950
Nadir range	500-12,300	400-6800
% with ≤ 2000	60	54
% with ≤ 1000	17	21
Thrombocytopenia		
No. of evaluable patients	65	56
Median nadir	139,000	124,000
Nadir range	51,000-216,000	25,000-304,000
% with ≤ 130,000	46	61
% with ≤ 75,000	8	11
Nonhematologic		
No. of evaluable patients	66	59
% with—		
Nausea		
Any	82	73
Severe	15	17
Emesis		
Any	58	58
Severe	14	14
Stomatitis		
Any	26	14
Severe	2	3

CAF (tamoxifen + CAF). Patients were eligible only at the time of their first recurrence, and prior treatment with adjuvant therapy was permissible only if it had been completed at least 6 months prior to entry in the study. The overall objective response rates (CR + PR) were 51% for CAF and 56% for T-CAF; projected median response durations were 14.6 and 17.3 months, respectively. Examinations of responses according to ER status were also presented. This study is notable in that 150 (65%) of the patients had the ER status known. There were no significant differences between the two regimens for either the ER-positive or -negative subsets. The response rate for T-CAF was almost twice that of CAF (65% vs 33%) in patients whose ER status was unknown. This finding is difficult to interpret given the findings within known ER subsets unless explainable by reasons such as noncomparability based on other factors. The investigators concluded that the addition of tamoxifen to CAF offers no therapeutic advantage regardless of ER status.

The findings of our study are of interest in light of the work by Lippman et al (14) that in MCF-7 human breast cancer cells, physiologic concentrations of estradiol stimulate cell division, whereas tamoxifen is inhibitory. In addition, they noted that estradiol was able to "rescue" cells previously incubated with tamoxifen so that thymidine incorporation rose to levels higher than those for both control and estradiol-treated cells. Osborne (15) has outlined the hypothesis that an interaction between hormonal therapy and chemotherapy in the clinical setting may result in an adverse, or at least less than maximally additive, effect on cell kill. Considering tamoxifen therapy specifically, he notes that a larger fraction of tumor cells are present in the G1 phase, suggesting a transition delay in the cell cycle. Since some cytotoxic agents are not effective against cells in a prolonged G1 phase, this alteration in cell kinetics may have a deleterious effect on the activity of chemotherapy.

Several groups of investigators have presented clinical data exploring attempts at hormonal synchronization. Lippman et al (16) reported the results of a clinical trial in which 110 patients were randomized to either four-drug chemotherapy alone (cyclophosphamide, doxorubicin, 5-FU, and methotrexate) or combined with tamoxifen and Premarin. The objective response rates (CR + PR) were identical (65%). The time to disease progression and survival of patients achieving a PR, however, significantly favored the chemotherapy/hormonal therapy regimen. Allegra et al (17), in a phase II trial, utilized 18-day cycles involving 10 days of CFP followed by 4 days of Premarin and then chemotherapy consisting of moderately high dose methotrexate followed by 5-FU and leucovorin rescue. Twenty-five patients were evaluable; the overall response rate was 72%. Of particular note is that 14 (56%) patients achieved CR. Although their patients had favorable characteristics, the high proportion of complete responders would justify further attention to this approach.

Our study revealed no indication of benefit from the addition of tamoxifen to a CFP chemotherapy regimen over that regimen utilized alone. The question of the value of tamoxifen as a synchronizing agent in a chemotherapy/hormonal therapy approach remains to be answered.

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Tamoxifen Therapy in Premenopausal Women With Metastatic Breast Cancer[1]

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Forty-three premenopausal women with metastatic breast cancer were treated with tamoxifen (2 × 20 mg daily) as first-line treatment. The patients were not selected on receptor status, which was known in only a few patients. Complete response was achieved in six patients and partial response was achieved in seven patients, for a total response rate of 30%. At the time of disease progression (including nonresponding patients), castration was performed as second-line treatment in 24 patients. Four of eight responders to tamoxifen had a response to castration. Of four patients with stable disease receiving tamoxifen, one had a response, and of 12 patients failing to respond to tamoxifen, one had a complete response and one had a partial response. In conclusion, patients who respond to tamoxifen have a good chance of responding to castration; an initial failure to tamoxifen does not exclude the possibility of response to castration. Therefore, the prognostic value of the tamoxifen response for castration response is of limited value. [Cancer Treat Rep 69:363–368, 1985]

Since the end of the 19th century castration has been the treatment of first choice in premenopausal patients with metastatic breast cancer. There is no difference between surgical and roentgen castration in terms of response rate or response duration (1,2). The average response rate is 30%, with a median duration of 12–18 months. In the case of estrogen receptor (ER)-positive tumors, the response rate rises to 50%–70% (1–3).

Because a great number of patients have had this therapy without any benefit, it would be desirable to find a factor predictive for the chance of response to castration, especially when the ER status is unknown.

Treatment results with tamoxifen in 167 premenopausal patients published in several series (4–14) have shown a response rate of 33% and a response duration similar to or sometimes better (8) than that of castration.

As in the postmenopausal patients, the response rate is higher in premenopausal women with ER-positive tumors (6,8). The fact that responses are also observed in ER-negative patients suggests that the action of tamoxifen is not solely by competitive inhibition of the ER of the tumor cell (15). The "antiestrogen binding site" found in in vitro experiments (16,17) could be the explanation of this observation, apart from a false-negative or a nonfunctioning receptor.

If the mechanism of action of tamoxifen is not only due to blocking of the ER (comparable to estrogen deprivation after oophorectomy) but also due to specific antiestrogenic activity, there should be no absolute cross-resistance between tamoxifen and castration in breast cancer.

Experiments in nude mice implanted with human ER-positive breast cancer showed no cross-resistance between tamoxifen and castration on tumor growth (18). The data about response to castration after tamoxifen therapy are sparse and not conclusive. In 13 patients who initially responded to tamoxifen, Manni and Pearson (6) and Pritchard et al (11) observed a response after castration in nine patients; however, none of the patients who failed to respond to tamoxifen obtained a response after castration, suggesting a predictive value of the tamoxifen treatment result. On the other hand, Hoogstraten et al (13) found in their series several responses after castration (while continuing tamoxifen) in patients who failed to respond to tamoxifen as first-line treatment. A castration response after tamoxifen treatment failure was also reported by Kalman et al (14).

In our institute a phase II study was performed with tamoxifen as first-line treatment in premenopausal women with metastatic breast cancer to determine the effec-

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TABLE 1.—Patient characteristics

Patient No.	Age (yrs)	Receptor status*	Disease-free interval (mos)	Site of dominant metastasis	Influence of tamoxifen on castration	Response to tamoxifen after 8 wks of treatment†	Time to disease progression (mos)	Second-line treatment	Response after castration†	Time to disease progression (mos)	Status
1	34	Unknown	3	Bone	Regular	SD	36+	—	—	—	Alive
2	50	Unknown	12	Soft tissue	Amenorrhea	PD	0	Castration	CR	27+	Alive
3	56	ER-/PR+	17	Soft tissue	Amenorrhea	PD	0	Castration	PR	21+	Alive
4	49	Unknown	9	Bone	Irregular	SD	24+	—	—	—	Alive
5	47	Unknown	27	Soft tissue	Regular	SD	6	Castration	SD	16+	Alive
6	51	Unknown	30	Soft tissue	Irregular	CR	4	Castration	PD	0	Alive
7	39	Unknown	90	Soft tissue	Irregular	PR	5	Castration	PR	24+	Alive
8	49	Unknown	62	Bone	Irregular	SD	29+	—	—	—	Alive
9	38	Unknown	0	Bone	Regular	SD	4	Castration + hormonal therapy	—	—	Alive
10	51	ER+/PR+	13	Soft tissue	Irregular	PD	0	Castration	SD	8	Dead
11	44	Unknown	7	Visceral	Irregular	PD	0	Chemotherapy	—	—	Dead
12	40	Unknown	27	Visceral	Regular	PD	0	Castration	PD	0	Dead
13	40	Unknown	44	Soft tissue	Regular	PD	0	Chemotherapy	—	—	Dead
14	49	Unknown	0	Soft tissue	Regular	PD	0	Hormonal therapy	—	—	Dead
15	39	Unknown	20	Visceral	Amenorrhea	PD	0	Hormonal therapy	—	—	Dead
16	32	Unknown	23	Visceral	Irregular	PD	0	Chemotherapy	—	—	Dead
17	34	Unknown	19	Visceral	Regular	PD	0	Castration	PD	0	Dead
18	47	Unknown	70	Soft tissue	Irregular	PR	20	Castration	CR	12+	Alive
19	36	Unknown	49	Visceral	Regular	PD	0	Castration	PD	0	Dead
20	43	Unknown	28	Bone	Irregular	PD	0	Castration	PD	0	Dead
21	44	Unknown	30	Soft tissue	Irregular	PR	10	Chemotherapy	—	—	Alive
22	31	Unknown	12	Visceral	Irregular	PD	0	Castration	PD	0	Dead
23	41	Unknown	72	Visceral	Amenorrhea	CR	23	Castration	PR	8+	Alive
24	35	ER+/PR-	14	Soft tissue	Irregular	CR	24	Castration	PD	0	Dead
25	46	Unknown	24	Soft tissue	Irregular	CR	36+	—	—	—	Alive
26	51	Unknown	33	Visceral	Amenorrhea	PD	0	Hormonal therapy	—	—	Dead
27	41	Unknown	9	Bone	Irregular	PD	0	Castration	SD	4	Dead
28	49	Unknown	47	Soft tissue	Uterine extirpation	CR	26+	—	—	—	Alive
29	28	Unknown	22	Soft tissue	Regular	SD	4	Castration	PR	8	Dead
30	42	Unknown	19	Soft tissue	Irregular	SD	7	Castration	PD	0	Alive
31	43	Unknown	7	Soft tissue	Amenorrhea	CR	12	Castration	PD	0	Alive
32	42	Unknown	13	Soft tissue	Regular	PD	0	Hormonal therapy	—	—	Dead

33	48	Unknown	19	Soft tissue	Amenorrhea	PD	0	Castration	PD	0	Dead
34	47	ER+/PR+	0	Soft tissue	Irregular	SD	5	Hormonal therapy	—	—	Alive
35	47	Unknown	30	Soft tissue	Regular	PR	6	Castration	PR	20+	Alive
36	47	Unknown	38	Visceral	Amenorrhea	PR	32+	—	—	—	Alive
37	51	Unknown	32	Soft tissue	Amenorrhea	PR	30+	—	—	—	Alive
38	47	ER-/PR-	24	Soft tissue	Regular	PD	0	Castration	PD	0	Dead
39	45	Unknown	19	Visceral	Irregular	SD	3	Castration	PD	0	Dead
40	40	ER+/PR+	2	Bone	Regular	PD	0	Castration	PD	0	Dead
41	53	ER+/PR-	14	Visceral	Amenorrhea	PR	5	Castration	PD	0	Dead
42	54	Unknown	36	Visceral	Amenorrhea	SD	27+	—	—	—	Alive
43	53	Unknown	17	Soft tissue	Amenorrhea	PD	0	Hormonal therapy	—	—	Alive

* PR = progesterone receptor.
†SD = stable disease; PD = progressive disease; CR = complete response; and PR = partial response.

tiveness of the treatment in this patient group and to determine whether the tamoxifen response has a predictive value for the response on subsequent castration.

MATERIALS AND METHODS

Forty-three consecutive premenopausal patients with evaluable measurable metastatic breast cancer were entered in the study during 1979–1981 and were treated with tamoxifen (2 × 20 mg daily) as first-line treatment. None of the patients had been treated with adjuvant chemotherapy. With the exception of one patient who had had a hysterectomy, all patients menstruated regularly at the start of treatment. Patient characteristics are shown in table 1. The median age at start of treatment was 45 years (range, 28–56); the median disease-free interval was 20 months (range, 0–90).

Evaluation of the treatment results took place after 8 weeks according to World Health Organization response criteria (19). In the case of CR, PR, or stable disease, therapy was continued until disease progression; in initial progressive disease or disease progression after tamoxifen response or stable disease, a castration was performed as second-line treatment when possible.

RESULTS

First-Line Treatment

All entered patients were evaluable for response to tamoxifen. CR was achieved in six of 43 patients (14%) and PR was achieved in seven of 43 patients (16%), with a median response duration of 23 months (range, 4–36+) and 10 months (range, 5–32+), respectively. Ten of 43 patients (23%) had stable disease, with a median time to disease progression of 6 months (range, 3–36+). Twenty of 43 patients (47%) had initial progressive disease. The overall response rate was 30%. The response rates related to the dominant sites of disease are shown in table 2.

Response was observed only in soft tissue and visceral metastases. However, all responding visceral metastasis was lung metastasis; no objective responses were seen in liver metastasis (three of 13 patients) or bone metastasis.

Response Rate in Relation to Disease-Free Interval

Response to tamoxifen was statistically significantly better when the disease-free interval was longer ($P < 0.05$; Spearman rank-correlation test). Of four patients with a disease-free interval > 5 years, three responded to tamoxifen; of 30 patients with a disease-free interval of 1–5 years, nine responded; and of nine patients with a disease-free interval < 1 year, only one responded.

TABLE 2.—Response rate to tamoxifen, related to dominant site of disease*

	Response†				
	CR	PR	SD	PD	Total
Soft tissue	5	5	4	9	23
Visceral	1	2	2	8	13
Bone	0	0	4	3	7
	6 (14%)	7 (16%)	10 (23%)	20 (47%)	43
Median response duration in mos (range)	23 (4-36)	10 (5-32)	6 (3-36)		

* Unless otherwise specified, values = No. of patients.
†*See* table 1 for definitions.

Influence on Menstrual Cycle

Irregular cycles occurred in 17 of 42 patients and amenorrhea occurred in 12 of 42 patients during tamoxifen treatment, while the other 13 patients continued menstruating regularly. In the group of patients with amenorrhea, five of 12 had a response; in the group with irregular cycles, six of 17 had a response; and in the regularly menstruating group, one of 13 had a response. The difference in the response rates between these groups is not statistically significant ($P > 0.05$; Spearman rank-correlation test).

As expected, the patients who continued menstruating regularly had a lower median age (40 years) than patients with irregular menstruation (44 years) or amenorrhea (51 years). The difference in the median ages between these groups is significant ($P = 0.02$; Spearman rank-correlation test).

Side Effects

Side effects were negligible; no hypercalcemia was found during treatment. Nine patients had complaints probably related to treatment: eight episodes of flushing; six episodes of "premenstrual" emotional tension; and five episodes of pain in the lower abdomen.

Second-Line Treatment

At the time of this report eight patients are still in remission while receiving tamoxifen. Castration as second-line treatment was performed in 24 patients (16 had oophorectomy; eight had roentgen castration). Six patients refused castration and were treated with other additive hormone therapy, while four patients with rapid progressive disease were immediately treated with chemotherapy. In one patient a protocol violation occurred. The patient was treated with a combination of castration and additive hormonal therapy. The responses to castration are shown in table 3.

Four of eight responders to tamoxifen achieved CR or PR on castration (median time of response duration, 20+ months; range, 8+ to 24+); of four patients with stable disease receiving tamoxifen, one achieved PR (20+ months). Of the 12 patients who failed to respond to tamoxifen treatment, one achieved CR and one achieved PR (21+ and 27+ months, respectively). Both patients had soft tissue metastasis. There is no significant difference in the chance of achieving a response to castration between tamoxifen responders and nonresponders in this patient group ($P < 0.05$; Spearman rank-correlation test).

DISCUSSION

Tamoxifen is an effective additional hormonal treatment of metastatic breast cancer and is widely used in postmenopausal patients as drug of first choice. Experience with tamoxifen in premenopausal patients was limited until now. Previously reported treatment results (4-13) showed that in this patient group tamoxifen was a

TABLE 3.—Response rate to castration in patients pretreated with tamoxifen*

	Response to castration†				
Response†	CR	PR	SD	PD	Total
CR		1		3	4
PR	1	2		1	4
SD		1	1	2	4
PD	1	1	2	8	12
Total	2	5	3	14	24

* Values = No. of patients. Spearman rank-correlation test: not significant.
†*See* table 1 for definitions.

safe and effective treatment, with treatment results comparable to those of castration. Our results support these findings. We observed a 14% CR rate and a 16% PR rate in our patient group, with a median response duration of 23 and 10 months, respectively.

The duration of the disease-free interval is an important prognostic factor, correlated with the chance of achieving a response with tamoxifen.

Irregular menstrual cycles and amenorrhea were found to be more frequent in the older women; no relation could be found, however, between arrest of menstruation by tamoxifen and the chance of response. The cessation of menstruation alone, especially in the older age group, may not be considered as a sign of a complete medical castration; for example, Patients 2 and 3 developed amenorrhea within 8 weeks of treatment without achieving a response, but did respond after castration.

The continuation of regular menstruation, on the other hand, suggesting an incomplete antiestrogenic effect at the level of the uterus, can occur in patients who respond to treatment, even despite high serum estrogen levels and high estrogen midcycle peaks as shown by Manni and Pearson (6) and Sherman et al (27).

Previous studies (6,10,11) suggested a cross-resistance between tamoxifen and castration. If this prediction is true a great number of patients could be spared castration. Recently (13,14), however, responses were reported after castration when there had been initial failure to tamoxifen, making cross-resistance unlikely. Our results are in conformity with these latter findings: of 12 patients who initially failed on tamoxifen, two obtained a response after castration. Of the eight patients who responded to tamoxifen, four responded after castration, a response in agreement with the observations of Manni and Pearson (6) and Pritchard et al (10,11).

The lack of cross-resistance between tamoxifen and castration can be explained if an incomplete antiestrogenic effect of tamoxifen is assumed. A second explanation could be an effect mediated by an independent "antiestrogen binding site" (16,17). Although the existence of antiestrogen binding sites is made plausible by several studies (19-23), their function, if any, is not yet elucidated (24-26). Further investigations will be needed to answer these questions.

CONCLUSION

Tamoxifen is an effective treatment in premenopausal metastatic breast cancer; the response rate and duration are comparable to those of castration. Patients who respond to tamoxifen have a fair chance of responding to castration when disease progression occurs. Failure on tamoxifen therapy does not exclude the possibility of obtaining a response to castration.

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Etoposide and Cisplatin Salvage Chemotherapy for Small Cell Lung Cancer[1,2,3]

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Thirty patients with previously treated small cell lung cancer received salvage combination chemotherapy with etoposide and cisplatin. Two complete and six partial responses were observed, for a major response rate of 27%. Responses occurred promptly and sustained palliation was achieved among responders. Myelosuppression was the major dose-limiting toxic effect. A schedule of etoposide (115 mg/m² iv on Days 1–3) and cisplatin (25 mg/m² iv on Days 1–3 every 28 days) is recommended for further clinical trials. [Cancer Treat Rep 69:369–371, 1985]

Small cell carcinoma of the lung accounts for approximately 20% of all lung cancers (1). Within the last 5 years, major emphasis has been placed on developing increasingly more complex combined modality protocols to increase the number of complete remissions and thus improve overall survival rates (1,2). There are a large number of agents with demonstrated efficacy in small cell lung cancer when used singly and in combination. Etoposide (VP-16) is perhaps the most promising of the newer drugs. Clinical experience in the United States and Europe with this podophyllotoxin has shown single-agent response rates ranging between 20% and 51% (3–5). Cisplatin has been less well evaluated against small cell lung cancer; response rates ranging between 0% and 12% have been reported (6,7). The combination of VP-16 and cisplatin has been shown to be synergistic against P388 leukemia and B16 melanoma (8). Furthermore, this combination was used by Sierocki et al (9) as initial therapy for small cell lung cancer with marked initial success (95% major response rate). We therefore investigated the use of this combination against recurrent or progressive disease in previously treated patients.

MATERIALS AND METHODS

Thirty consecutive patients with histologically confirmed small cell lung cancer showing evidence of disease progression are reported. Eligibility criteria included failure of conventional chemotherapy to induce an initial response within two cycles of treatment or failure of chemotherapy to maintain an initial response; no prior exposure to either of the study drugs; presence of measurable disease; ambulatory status; and adequate renal, hepatic, and bone marrow functions unless involved by tumor. An IRB-approved written consent form was obtained from all patients. Pretreatment patient characteristics are shown in table 1. No patient had been off treatment for lung cancer for > 4 weeks.

At time of entry, six patients had limited disease and 24 had extensive disease. Of the latter group, 14 patients had presented with limited disease at the time of initial diagnosis.

Three treatment regimens were used. The regimen for the first eight patients consisted of VP-16 at a dose of 150 mg/m² given iv over 30 minutes on Days 1–3 and cisplatin at a dose of 20 mg/m² given by iv push on Days 1–5 of each 28-day cycle. The regimen for the next 15 patients consisted of drug doses reduced to 115 and 15 mg/m² for VP-16 and cisplatin, respectively. In the last seven patients cisplatin was administered at 25 mg/m² for 3 successive days. All patients were hospitalized for therapy. Hydration for cisplatin was 1 L of normal saline within 12 hours of starting treatment, and normal saline at 100–125 ml/minute was maintained throughout the hospital stay. No furosemide or mannitol was routinely used. Antiemetic prophylaxis was given with either perphenazine or metoclopramide.

Drug dose modifications were as follows. For a total wbc count of 2500–3000/μl and/or a platelet count of 75,000–100,000/μl on the day of therapy, both drugs

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TABLE 1.—Patient characteristics and responses*

		Response to VP-16/cisplatin			
	Total	Complete	Partial	Minor	Disease progression
No. of patients	30	2	6	7	15
Median age in yrs (range)	59 (45-76)	63	58	60	57
Sex					
Male	16	2	2	5	7
Female	14	0	4	2	8
Eastern Cooperative Oncology Group performance status					
1	12	2	4	4	2
2	15	0	1	3	11
3	3	0	1	0	2
Median duration of disease in mos (range)	10 (2-34)	9	14	10	8
Prior therapy					
Surgery					
Lobectomy	1	0	0	1	0
Radiation therapy					
Mediastinum	14				
Cranium	12				
Chemotherapy†					
CAV	23	1	4	6	12
POCC	6	1	2	2	1
MACC	1	0	0	0	1
Sites of metastases					
Lung	24	1	5	4	14
Liver	9	1	1	1	6
Bone	7	0	0	1	6
Pleural effusion	5	1	1	0	3
Lymph nodes	4	0	0	1	3
Skin, soft tissue	3	0	0	2	1
Bone marrow	1	0	0	0	1
Brain	1	0	0	0	1
Median survival (mos)					
From diagnosis	13	20	18	15	12
From VP-16/cisplatin	4	12	6	7	3

* Unless otherwise specified, values = No. of patients.

†CAV = cyclophosphamide, doxorubicin, and vincristine; POCC = procarbazine, vincristine, cyclophosphamide, and lomustine; and MACC = methotrexate, doxorubicin, cyclophosphamide, and lomustine.

were withheld for 1 week. For a wbc count of $< 2500/\mu l$ and/or a platelet count of $< 75{,}000/\mu l$, both drugs were withheld until the wbc count was $\geq 3000/\mu l$ and/or the platelet count was $> 100{,}000/\mu l$, at which time VP-16 was reinstituted at 50% of calculated dose for that particular cycle and cisplatin was given at full initial dose. Cisplatin was withheld if creatinine was > 2.0 mg/dl or if creatinine clearance fell to < 40 ml/minute.

A total of 102 cycles were administered (range, one to 12 cycles/patient). Five patients received only one cycle; excluding these, the median number of cycles was 3.5 (mean, 4.3).

Complete response (CR) was defined as the disappearance of all clinical evidence of tumor for at least 4 weeks. Partial response (PR) was defined as a > 50% decrease in the sum of the products of the perpendicular diameters of measured lesions without an increase in the size of the lesions or the appearance of new lesions. Minor response was defined as less than PR and no worsening of symptoms, for a minimum of 2 months. Disease progression was defined as an unequivocal increase of > 25% of any measurable lesions or the appearance of new lesions.

RESULTS

All 30 patients have been followed until death and are evaluable for response. Two CRs and six PRs were observed, for a major response rate of 27%. Seven other patients achieved minor responses. Responses were observed among the three treatment regimens: in three of eight patients with the initial higher dose 5-day schedule; in three of 15 including both complete responders with

the lower dose 5-day schedule; and in two of seven with the 3-day schedule. Among the six patients with limited disease, three PRs were seen. Of 14 patients with limited disease at diagnosis and extensive involvement by cancer at time of entry in our study, one CR and two PRs were achieved. Finally, among the ten patients with extensive disease at diagnosis, one CR and one PR were achieved. Therefore, six of eight major responses were observed in the population of patients with limited disease at diagnosis. Of the two patients achieving CR with the salvage regimen, one had previously obtained a CR with CAV plus radiation therapy given prophylactically to the central nervous system. The other patient had shown no response after two cycles of POCC. Of six patients achieving PR with VP-16 and cisplatin, two had previously achieved CR with CAV plus irradiation, three had previously achieved PR (two with CAV and one with POCC), and one was a nonresponder to POCC.

Toxic effects included mild nausea and vomiting, which was usually well-controlled by the addition of methylprednisolone to the antiemetic regimen. Myelosuppression was universal, with the first eight patients requiring a dose modification in the study design, as previously mentioned. Twenty-seven patients are evaluable for toxicity. The remaining three patients died within 3 weeks of therapy, without evidence of drug toxicity. Sixteen patients had wbc count nadirs of $< 2900/\mu l$. Five of these patients had wbc count nadirs between 1000 and $1900/\mu l$. Thrombocytopenia was less common. With the first treatment regimen two patients had platelet count nadirs of 2000 and $19{,}000/\mu l$, respectively. Platelet counts $< 75{,}000/\mu l$ were not seen with the other two schedules. Myelosuppression was reversible, allowing for cycles to be administered at 28-day intervals. Three patients became bacteremic while neutropenic; one of these patients died with septicemia and bleeding 2 weeks after the first course of high-dose 5-day VP-16/cisplatin. In two patients, renal toxicity required permanent discontinuation of cisplatin. One patient developed irreversible sensory motor neuropathy involving tibial and peroneal nerves after having six cycles of treatment. One patient had clinically apparent hearing loss. One patient had a mild allergic reaction resulting in periorbital edema probably secondary to cisplatin.

DISCUSSION

The observed major response rate of 27% in this group of previously treated patients confirms the therapeutic activity of VP-16/cisplatin against small cell lung cancer as described by Sierocki et al (9) and Madrigal et al (10). The observed median survival from diagnosis of 18 months for our group of patients who responded to the salvage program compares favorably with survival rates obtained by much more aggressive programs. Furthermore, recent preliminary communications utilizing this same combination for salvage therapy appear to confirm the therapeutic activity observed by us (11). The median survival among minor responders of 15 months from diagnosis and 7 months from VP-16/cisplatin also deserves comment. Seven patients received a median number of five cycles of chemotherapy. The duration of response was therefore about 5 months. In this group, enough of a tumor response was achieved to allow for survival which was considerably longer than that observed with patients in whom no response or obvious disease progression occurred. This latter group of patients had a median survival of only 3 months from beginning of VP-16/cisplatin.

Much emphasis is being placed on clinically verifying the ability of cisplatin to synergize in animal models with a variety of antineoplastic agents such as carmustine, cytarabine, 5-FU, and VP-16 (8). The present study was not designed to evaluate such a synergism but the observed objective responses in this poor prognostic group of patients in this series do suggest an enhanced therapeutic effect for the combination. In summary, VP-16/cisplatin can provide effective palliation in patients who have failed initial chemotherapy and irradiation. Administration of cisplatin over 3 days appears to be as effective as administration over 5 days, with the added advantage that hospitalization is significantly shortened. Toxicity of this regimen is only moderate with the 3-day dose and schedule proposed.

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Failure of Scalp Hypothermia To Prevent Hair Loss When Cyclophosphamide Is Added to Doxorubicin and Vincristine[1]

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Scalp hypothermia can prevent alopecia caused by low doses of doxorubicin alone or in simple combinations. The technique was used in 60 patients with breast cancer (24 receiving adjuvant therapy; 36 with advanced recurrent disease) receiving chemotherapy with iv doxorubicin (40 mg/m^2) and vincristine (1.4 mg/m^2) on Day 1 together with oral cyclophosphamide (200 mg/m^2) on Days 2–5. The patients' desire to continue scalp hypothermia, reflecting their perception of benefit, and an objective assessment of hair retention were the study end points. The mean number of cycles of chemotherapy given (6.1 in patients receiving adjuvant therapy; 3.8 in those with advanced disease) exceeded the number of cycles with hypothermia (2.1 in patients receiving adjuvant therapy; 1.6 in those with advanced disease); no patients retained enough hair to encourage them to continue scalp hypothermia throughout chemotherapy. All patients were rated as having poor hair retention. Scalp hypothermia is ineffective when used with combinations of drugs, each causing alopecia, or with high doses of doxorubicin. [Cancer Treat Rep 69:373–375, 1985]

Alopecia is one of the most distressing consequences of cancer chemotherapy. Doxorubicin nearly always causes severe hair loss so that a wig is temporarily required. Recently, several studies have shown that lowering the temperature of the scalp with cryogel ice packs can markedly decrease the incidence of severe hair loss. It is thought that the induced hypothermia reduces scalp blood flow, preventing access of the drug to the hair follicles. Only a short period of cooling is required since doxorubicin is rapidly cleared from the plasma.

Most successful studies of scalp hypothermia have used relatively low doses of doxorubicin (1,2), alone or with drugs that do not cause alopecia (3). Current cancer chemotherapy frequently includes combinations of drugs, each capable of causing a degree of alopecia, and this study was designed to see how effective scalp cooling would prove when used with such a drug combination. The process of scalp cooling is time consuming, relatively expensive, and can be uncomfortable so that therapeutic benefit is a balance between this and the degree of hair retention as perceived by the patient.

METHODS

Sixty patients receiving chemotherapy for breast cancer were entered in the study. Characteristics of patients undergoing scalp hypothermia are as follows. Twenty-four patients had adjuvant therapy for T1-2 N0 M0 estrogen receptor (ER)-negative disease or T1-2 N1 M0 ER-positive or -negative disease. Thirty-six patients had recurrent metastatic disease; 30 had received prior hormonal therapy, and none had had prior chemotherapy. Chemotherapy used for adjuvant treatment and for advanced disease included doxorubicin (40 mg/m^2 iv, Day 1), vincristine (1.4 mg/m^2 [maximum, 2.0 mg] iv, Day 1), and cyclophosphamide (200 mg/m^2 orally, Days 2-5). This was repeated every 3 weeks × 8. If the wbc count was < 3000 × 10^9/L or the platelet count was < 100,000 × 10^9/L on Day 22, therapy was delayed by 1 week and subsequent therapy was given at 4-week intervals. All patients had a normal growth of head hair prior to chemotherapy.

The method of scalp cooling used for the first 30 patients was identical to that described in a previous study (4). Patient hair was soaked and a wet crepe bandage wrapped "turban style" around the head to improve conduction. Six prefrozen cryogel packs were applied to the scalp and held in place with a perspex helmet. The remaining 30 patients received scalp cooling using a commercially available technique, the Spenco Hypothermia

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Cap (Spenco Medical Corp, Waco, TX). This was placed directly onto the patients' wet hair and was bandaged into place. Whichever technique was used, the scalp was allowed to cool for 20 minutes before an iv injection of doxorubicin was given. Cooling was continued for a further 30 minutes and the cryogel packs or hypothermia cap was then removed. Scalp temperature, measured by thermocouples, was recorded in a previous study (4) and showed that these techniques give reproducible lowering of scalp temperature which correlated with hair retention.

Measurement of hair retention is essentially subjective and because of this the patients' desire to continue scalp cooling was selected as an end point of the study. Patients abandoning scalp hypothermia while continuing planned chemotherapy were scored as therapeutic failures. In addition, objective assessment of the degree of scalp hair retention was made by the nurse coordinating the project. Protection from hair loss was rated as good (< 33% loss), moderate (33%-66% loss), or poor (> 66% loss).

RESULTS

Patients having adjuvant chemotherapy (table 1) received a mean of 6.1 ± 2.3 cycles of chemotherapy (range, one to eight), while those given chemotherapy for metastatic disease received a mean of 3.8 ± 2.3 cycles (range, one to eight). In the group of 24 patients receiving adjuvant therapy, none wished to continue scalp hypothermia throughout their course of chemotherapy and all were scored as failures. The mean number of cycles (table 1) of adjuvant chemotherapy which included scalp hypothermia was 2.1 ± 1.7 cycles (range, one to seven). Similarly, all patients undergoing scalp cooling during chemotherapy for advanced disease were regarded as therapeutic failures, having abandoned hypothermia during chemotherapy. The mean number of cycles including scalp cooling in this group was 1.6 ± 0.95 cycles (range, one to five). Overall, none of the 60 patients had sufficient hair retention to encourage them to persist with scalp hypothermia throughout chemotherapy. Objectively, all patients were rated as having had poor hair retention (> 66% hair loss), and all required a wig at the time they abandoned scalp hypothermia.

Four patients developed scalp metastases after one, one, two, and three cycles. Each of these patients had lost significant hair and hypothermia was discontinued. All patients with scalp nodules had advanced recurrent disease so that the risk of scalp metastases during scalp cooling was 11% for this group. There have been no scalp relapses in the patients receiving adjuvant therapy.

TABLE 1.—No. of cycles including scalp hypothermia in patients receiving chemotherapy

Chemotherapy group (No. of patients)	Mean No. of chemotherapy cycles ± SD	Mean No. of cycles including hypothermia ± SD
Adjuvant therapy (24)	6.1 ± 2.3	2.1 ± 1.7
Advanced disease (36)	3.8 ± 2.3	1.6 ± 1.0

DISCUSSION

Previous studies (1,3-6) have shown that a proportion of patients who undergo scalp hypothermia when they receive doxorubicin have useful preservation of hair. The degree of retention is dependent on the dose of doxorubicin used (5), the minimum scalp temperature achieved (4), and possibly on the presence of liver impairment (6). This study reports the effect of scalp hypothermia in a group of patients receiving a combination of drugs, all of which are capable of causing differing degrees of alopecia.

The pharmacokinetics of vincristine (7) and doxorubicin (8) are such that high peak levels in the serum persist for a relatively short period, < ½ hour. However, low levels of doxorubicin may be present for many hours after injection (9). In contrast, the activation and metabolism of cyclophosphamide are much slower (10), so that a short period of scalp hypothermia (30 minutes) would not prevent the access of active metabolites to scalp hair follicles. Despite the likelihood that hypothermia would fail to prevent cyclophosphamide-induced alopecia, it was decided to use the technique since hair loss is not usually severe at the dose of cyclophosphamide used.

In addition to a lack of effect against cyclophosphamide, the low levels of doxorubicin that persist in plasma for long periods after injection may be sufficient to cause some hair loss. This may well be additive when other drugs are given.

In contrast with other studies, including our own in which 36% of patients retained hair after receiving the same doses of doxorubicin and vincristine together with prednisolone (4), none of the patients undergoing scalp cooling had sufficient benefit to encourage continued use of the technique and all had objective evidence of severe hair loss and required a wig. There was no evidence that liver function affected the outcome; all patients receiving adjuvant therapy had normal liver function and failed to benefit from hypothermia.

Since the only difference between this and our previous study was the substitution of cyclophosphamide for prednisolone, it seems highly likely that the introduction of this drug into the regimen led to severe alopecia. Although other moderately successful studies (1,5) have used combinations of doxorubicin with cyclophosphamide, they have used lower doses of each drug and have not included vincristine. The degree of alopecia was more than that usually seen with cyclophosphamide alone and may have resulted from the unopposed action of the cyclophosphamide together with the partially suppressed effects of doxorubicin and vincristine. The finding of

metastatic breast cancer in the scalp of four patients (11%) is worrying and argues strongly against the use of scalp cooling in patients with advanced metastatic cancer that may spread to the skin or soft tissues (2). The total failure of scalp hypothermia to usefully prevent alopecia in patients receiving this combination of chemotherapy suggests that it is unlikely to be beneficial in patients receiving many current chemotherapy combinations which contain maximal doses of cyclophosphamide together with vincristine and doxorubicin. The technique is cumbersome, expensive, time consuming, and often uncomfortable and cannot be recommended for routine use in these types of combinations.

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Clinical Trial Depleting T Lymphocytes From Donor Marrow for Matched and Mismatched Allogeneic Bone Marrow Transplants[1,2]

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Nine patients received T-lymphocyte-depleted histocompatible bone marrow and 28 patients received T-lymphocyte-depleted histoincompatible bone marrow. Eight of nine patients receiving matched bone marrow quickly engrafted without severe graft-versus-host disease (GvHD). None of the eight patients received anti-GvHD prophylaxis medications. Two of these eight patients are currently alive. Nonengraftment and severe GvHD were problems seen in some of the patients given the histoincompatible bone marrow. Additional cytarabine pretransplant permitted engraftment in those patients undergoing histoincompatible transplants for treatment of malignancy, and prednisone and cyclosporine posttransplant reduced the incidence of acute GvHD in those given T-lymphocyte-depleted grafts. Seven of these 28 patients are currently alive. T-lymphocyte-depleted marrow can reduce the occurrence or prevent severe acute GvHD, especially when combined with additional prednisone and cyclosporine; however, the impact on relapse patterns and survival remains to be determined. The occurrence of nonengraftment and treatment-related lymphomas are formidable problems to overcome. [Cancer Treat Rep 69:377–386, 1985]

Bone marrow transplantation (BMT) has an established place in the management and treatment of patients with acute leukemias, aplastic anemia (AA), immunodeficiency syndromes, and chronic myelogenous leukemia (CML) (1–11). However, its widespread use has been limited by two major problems: (*a*) the lack of histocompatible donors for many patients whose disease might respond to intensive chemotherapy-radiotherapy; and (*b*) the high incidence of acute graft-versus-host disease (GvHD) following matched transplants, limiting the availability of BMT to centers with the personnel and expertise to care for the patient with GvHD (12–15).

Many different chemotherapeutic and immunomodulating agents have been used to diminish or prevent GvHD in transplants that are HLA-mixed lymphocyte culture (MLC)-compatible as well as in mismatched transplants (15–25). Despite these measures, adverse effects are frequent and none of these drugs or antibody preparations has appeared to significantly decrease the incidence of GvHD (grades II–IV) below that of 40%–50% (15). A more recent technique, helpful in preventing GvHD in mouse transplant models when a bone marrow graft was histoincompatible (26), involves depleting immunocompetent T lymphocytes from donor bone marrow using antibodies to the T lymphocytes with and without complement. Several centers have treated donor bone marrow in vitro with OKT3 in the matched BMT setting (20,21) or have used lectin binding as a physical means of depleting T lymphocytes (22,23). However, except for one series from London where the incidence of acute GvHD was 18% (20), the results of these manipulations have not been encouraging in treating AA and hematologic malignancy, although three patients with combined immunodeficiency significantly benefited from lectin-separated marrow

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transplants from parents (22). A single report of monoclonal antibody treatment of maternal marrow suggested that engraftment with immunocompetent cells was possible (19). Of interest in this case was the occurrence of extremely severe GvHD. However, in vivo administration of the monoclonal antibody controlled the symptoms.

We previously reported one patient who underwent successful BMT without developing GvHD using histocompatible marrow depleted of T lymphocytes (27,28). Since then, we have used CT-2, a monoclonal antibody directed against the E-rosette receptor, combined with complement to deplete bone marrow of T lymphocytes prior to BMT. Our objectives were: (*a*) to extend the application of BMT to include those with histoincompatible donors; (*b*) to reduce or eliminate acute GvHD and to eliminate all agents used for GvHD prophylaxis; and (*c*) to begin to study the effects of receiving T-cell-depleted marrow including engraftment and graft versus leukemia phenomenon by assessing long-term survival and relapse patterns in those reconstituted with T-lymphocyte-depleted bone marrow.

We report our results in 37 patients who had either acute leukemia, CML, AA, immunodeficiency syndromes, or non-Hodgkin's lymphoma.

MATERIALS AND METHODS

Patients

Clinical data for the 37 patients are given in tables 1-3. Three patients had AA; 12 patients had acute lymphocytic leukemia (ALL) in first, second, or third remission; two patients had CML in first chronic phase; one child had juvenile CML in accelerated phase; four patients had CML in accelerated phase, blast crisis, or second chronic phase; one adult had relapsed T-cell lymphoma; four children had severe combined immunodeficiency (SCID); one child had Wiskott-Aldrich (WA) syndrome; and nine patients had acute myeloid leukemia (AML) in first or second remission except for Patient 037-23, who had an M2A marrow pre-BMT. All patients were accepted for BMT because of the poor prognosis associated with their diseases and all gave written informed consent for BMT and for the donor bone marrow to be depleted of T lymphocytes as described in protocols approved by the Human Subjects Committee of the University of Wisconsin.

Twenty-eight patients were given bone marrow from HLA-MLC mismatched donors with various degrees of phenotypic identity and all except Patient 026-12 had positive MLC reactivity. All of the donors for the mismatched transplants, except Patient 036-22, were related to the recipients. Nine patients were given HLA-MLC identical bone marrow from sibling donors as shown in table 1. None of the patients receiving sibling-matched (HLA A, B, C, D-identical and MLC-nonreactive) bone marrow were given methotrexate or other medication to prevent graft rejection or GvHD; however, Patient 033-19, with AA, received ATG and prednisone after her second transplant to prevent rejection. Five of the 28 mismatched patients received cyclophosphamide (2.5-7.5 mg/kg every other day) and prednisone (1 mg/kg/day) posttransplant to prevent graft rejection and GvHD (Patients 025-11, 031-17, 035-21, 036-22, and 037-23). Patients 038-24 and 041-27 to 054-37 (11 patients) who received T-cell-depleted bone marrow (table 3) received 4 mg/kg/day of cyclosporine (CsA) as a continuous iv infusion and 1 mg/kg/day of prednisone beginning on the day of marrow infusion, gradually tapering after the initial 3-4 weeks post-BMT. Several patients received more than one marrow infusion, as indicated in tables 1-3.

BMT Conditioning

The conditioning regimens used for the 37 patients are detailed in tables 1-3. All patients with AA re-

TABLE 1.—Clinical data on 9 patients with AA or leukemia who underwent BMT and had a matched donor

Unique Patient No.	Diagnosis	Age (yrs)/sex	Donor*	HLA-MLC	Disease status pre-BMT	Preparative regimen (initial regimen)†
012-01	ALL	8/M	S	Match	2nd remission	C, TBI-1
016-02	AML	19/M	S	Match	1st remission	C, TBI-2
019-05	ALL	29/M	S	Match	2nd remission	N, TBI-2
021-07	CML	26/M	B	Match	1st chronic phase	C, TBI-2
029-15	CML	22/M	B	Match	1st chronic phase	C, TBI-2
030-16	ALL	17/F	S	Match	3rd remission	D, C, TBI-1
032-18	ALL	26/F	S	Match	1st remission	C, TBI-2
033-19	AA	10/F	S × 2‡	Match	Not applicable	C*, TBI-3§
042-28	ALL	22/M	S	Match	2nd remission	A-2, C, TBI-1

*S = sister; and B = brother.

†C = cyclophosphamide (60 mg/kg × 2 doses); TBI = total-body irradiation (TBI-1, 1320 Rad given in eight 165-Rad fractions; TBI-2, 1200 Rad given in five 240-Rad fractions); N = mechlorethamine (1.5 mg/kg × 3 doses); D = daunorubicin (40 mg/m^2 × 2 doses); C* = cyclophosphamide (50 mg/kg × 4 doses); TBI-3 = 300 Rad given in two 150-Rad fractions; and A-2 = cytarabine (3 g/m^2 × 6 doses over 72 hrs).

‡Two bone marrow infusions, each from the same donor.

§Patient was given 2nd course of cyclophosphamide, antithymocyte globulin (ATG), and prednisone for 2nd marrow infusion.

ceived 300 Rad of TBI in addition to 200 mg/kg of cyclophosphamide. Patients with malignancies received cyclophosphamide (60–120 mg/kg); in some patients cytarabine, either as a continuous infusion (200 mg/m^2) or in intermittent doses of 3 g/m^2, was given. One patient received daunorubicin instead of cytarabine. One patient had long-standing cardiac toxicity documented prior to BMT and was given mechlorethamine instead of cyclophosphamide. The children with immunodeficiency syndromes requiring ablation prior to transplant were given busulfan (16 mg/kg) in addition to the cyclophosphamide without TBI.

Fractionated TBI was administered at a dose of 1200 Rad (240 Rad × 5 fractions) or 1320 Rad (165 Rad × 8 fractions) at a rate of 5 Rad/minute. Pretransplant intrathecal therapy consisted of cytarabine or methotrexate for patients with leukemia. No posttransplant intrathecal therapy was given to these patients.

Supportive Care

Isolation consisted of handwashing and face masks (no patient was kept in a laminar air-flow room), and the only special dietary precaution was the elimination of fresh fruits and vegetables. All patients received prophylactic ketoconazole and trimethoprim-sulfamethoxazole. Broad-spectrum antibiotics were instituted for fevers and infections and amphotericin B was used for documented fungal infections or persistent fever in a neutropenic patient. All blood products were irradiated and were given to maintain hemoglobin concentrations > 8 g/dl and platelet counts > 20,000/mm^3. Right atrial catheters were placed in all patients.

Bone Marrow Harvest

Bone marrow was aspirated from the anterior and posterior iliac crests of the anesthetized donor. The bone marrow was mixed with RPMI-1640 and glutamine (GIBCO, Grand Island, NY) plus heparin (50 units/ml). The aspirated volumes ranged from 300 to 1500 ml and were placed into 600-ml Fenwal blood bags. Details of these bone marrow techniques have been published (28).

T-Cell Depletion of Bone Marrow

CT-2 is an IgM mouse monoclonal antibody to the E-rosette receptor on T lymphocytes and has been found to be nontoxic to myeloid and erythroid precursors when normal bone marrow is treated in the presence of rabbit complement. Each lot of complement used for individual donor marrow treatment was screened on a sample of donor marrow 2–4 weeks before the scheduled transplant, as previously published (28).

The Fenwal bags of bone marrow were centrifuged to remove the plasma and fat and the buffy coat cells were then concentrated. CT-2 antibody was added to the buffy coat cells, and following a 30-minute incubation, baby rabbit serum was added. After an additional 60-minute incubation, the excess antibody and complement were removed and the bone marrow cells were resuspended in donor plasma and infused into the recipients over a 2–3-hour period through the central venous catheter. Details of the antibody and complement treatment of the bone marrow to remove the T lymphocytes are described elsewhere (28). The number of E-rosette-

TABLE 2.—Clinical data on 5 patients with SCID or WA who underwent BMT and had a mismatched donor

Unique Patient No.	Diagnosis	Age (yrs)/sex	Donor*	HLA-MLC†	Disease status pre-BMT‡	Preparative regimen (initial regimen)§
023-09	SCID	1/M	Fa × 2¶	A, B, C, haploidentical; MLC+	N/A	None for BMT-1, B, C* for BMT-2
026-12	WA	3 mos/M	Fa	A haploidentical; MLC–	N/A	B, C*
027-13	SCID	4/M	Fa	A, B, C, haploidentical; MLC+	N/A	None
028-14	SCID	1/M	Fa	A, B, C, haploidentical; MLC+	N/A	None
034-20	SCID	4/M	Fa	B haploidentical; MLC+	N/A	B, C*

*Fa = father.

†MLC+ = reactive in mixed lymphocyte culture; and MLC– = nonreactive in mixed lymphocyte culture.

‡N/A = not applicable.

§B = busulfan (4 mg/kg × 4 doses orally); and C* = cyclophosphamide (50 mg/kg × 4 doses).

¶Two bone marrow infusions, each from the same donor.

TABLE 3.—Clinical data on 23 patients with AA, leukemia, or lymphoma who underwent BMT and had a mismatched donor

Unique Patient No.	Diagnosis	Age (yrs)/sex	Donor*	HLA-MLC†	Disease status pre-BMT	Preparative regimen (initial regimen)‡
017-03	CML-juvenile	2/F	Fa	A, B, C haplo-identical; MLC+	Accelerated phase	C, A-1, TBI-1
018-04	AML	27/M	S	B haplo-identical; MLC+	1st remission	C, TBI-2
020-06	ALL	8/M	S × 2§ Fa × 1	HLA match; MCL+ A, B, C, haplo-identical; MLC+	3rd remission	C, TBI-1
022-08	AML	29/F	B × 2¶	A, B, C, haplo-identical; MLC+	3rd remission	C, A-2, TBI-2
024-10	ALL	39/F	S	HLA match; MLC+	1st remission	C, TBI-2
025-11	AA	22/M	B × 2¶	A, B, C haplo-identical; MLC+	Not applicable	C*, TBI-3
031-17	AML	26/F	B	A, B haplo-identical; MLC+	3rd remission	C, TBI-2
035-21	T lymphoma	41/M	S	A haplo-identical; MLC+	Active disease	A-2, C, TBI-2
036-22	AA	30/M	U	HLA match; MLC+	Not applicable	C*, TBI-3
037-23	AML	14/F	F	A, B haplo-identical; MLC+	M2A marrow, 2nd remission	C, TBI-1
038-24	ALL	10/M	Fa	A, B, C haplo-identical; MLC+	3rd remission (1 CNS relapse)	A-2, C, TBI-1
039-25	ALL	35/F	S	A, B, C match; MLC+	1st remission	A-2, C, TBI-1
040-26	AML	16/M	B	A, B, C haplo-identical; MLC+	2nd remission	A-2, C, TBI-1
041-27	CML	9/M	Mo	A, B, C, haplo-identical; MLC+	2nd chronic phase	A-2, C, TBI-1
043-29	ALL	14/M	Mo	A, B, C haplo-identical; direct match, MLC+	3rd remission (1 CNS, 1 testes relapse)	A-2, C+, TBI-1
044-30	CML	36/M	Son	B haplo-identical; MLC+	Accelerated phase	A-2, C, TBI-1
045-31	ALL	8/M	Fa	A, B, C haplo-identical; MLC+	2nd remission	A-2, C+, TBI-1
047-32	CML	19/M	Mo	A, B, C haplo-identical; MLC+	2nd chronic phase	A-2, C+, TBI-1
048-33	AML	15/M	B	A, B, C haplo-identical; MLC+	4th remission	A-2, C+, TBI-1
049-34	AML	14/F	Mo	A, B, C haplo-identical; MLC+	3rd remission	A-2, C+, TBI-1
051-35	CML	43/F	B	HLA match; MLC+	Blast crisis	A-2, C+, TBI-1
052-36	AML	4/F	Fa	A, B, C haplo-identical; MLC+	2nd remission	A-2, C+, TBI-1
054-37	ALL	7/F	Mo	B, C haplo-identical	3rd remission (1 CNS relapse)	A-2, C′, TBI-1

*Fa = father; S = sister; B = brother; U = unrelated donor; and Mo = mother.

†MLC+ = reactive in mixed lymphocyte culture; and MLC- = nonreactive in mixed lymphocyte culture.

‡C = cyclophosphamide (60 mg/kg × 2 doses); A-1 = cytarabine (200 mg/m^2 over 24 hrs × 2 doses); TBI-1 = 1320 Rad given in eight 165-Rad fractions; TBI-2 = 1200 Rad given in five 240-Rad fractions; A-2 = cytarabine (3 g/m^2 × doses over 72 hrs); C* = cyclophosphamide (50 mg/kg × 4 doses); TBI-3 = 300 Rad given in two 150-Rad fractions; C+ = cyclophosphamide (45 mg/kg × 2 doses); and C′ = cyclophosphamide (30 mg/kg × 2 doses).

§Two bone marrow infusions, each from the same donor, and 1 infusion from a 2nd donor; no repeat preparation treatment given for the 2nd and 3rd infusions.

¶Two bone marrow infusions, each from the same donor; no repeat preparation treatment for the 2nd infusion.

positive cells and OKT3-positive cells pre- and post-treatment are shown in tables 4-6. The dose of nucleated bone marrow cells remaining after treatment is also given in these tables. OKT3 reactivity is not inhibited by CT-2 treatment of cells and is used to determine the extent of depletion (28).

Assessment of Engraftment and GvHD

We defined the day of engraftment as the third consecutive day on which the wbc count was > 1000/mm^3 and increasing (21). Whenever possible, we documented engraftment of the donor marrow with karyotypic analysis of bone marrow cells, HLA phenotyping of peripheral blood lymphocytes, or rbc phenotyping. Two long-term survivors (Patients 020-06 and 024-10) had evidence of autologous recovery (table 6) and are the subjects of a more extensive analysis of host versus graft immunity.

We defined the severity of GvHD by the well-established Seattle criteria (12). GvHD was initially treated with prednisone (1-2 mg/kg/day) in those patients not already receiving prednisone, and, if unresponsive, then the dose was increased to 500-1500 mg/day. Patients already receiving prednisone were given 2 mg/kg/day, and, if unresponsive, then the dose was further increased. CsA, given at a dose of 5 mg/kg/day to Patient 035-21 for well-established GvHD, resolved all signs of GvHD.

RESULTS

The clinical outcome of the 37 patients who received allogeneic T-lymphocyte-depleted bone marrow is shown in tables 4-6. All patients were followed for at least 30 days posttransplant or until death occurred.

HLA-Matched Recipients

Nine patients with fully matched donors were entered in this study but only eight patients received fully matched allogeneic bone marrow transplants. One patient (No. 042-28) died prior to BMT from cardiac failure. Seven of the eight patients were leukemic and engrafted promptly; Patient 033-19 with AA had evidence of trilineage engraftment by 14 days after BMT but rejected the graft over the ensuing 7 days, necessitating a second transplant. Engraftment occurred 38 days after that second transplant. The mean time to engraftment was 23 days in all eight patients following BMT.

TABLE 4.—Clinical outcome for 9 patients with AA or leukemia and a matched donor

Unique Patient No.	Bone marrow dose (× 10^8 nucleated cells/kg)	E-rosette-positive cells		OKT3 cells		Day of engraftment†	GvHD‡	Outcome
		Pre*	Post	Pre	Post			
012-01	3.17	24	0	—	—	16	0	Relapsed on Day 199; died on Day 219
016-02	0.84	28.5	0	—	—	25	0	Died on Day 180, pneumonia§
019-05	1.33	28	0	—	—	31	I	Relapsed on Day 146; died on Day 181
021-07	4.06	22.4	0.4	—	—	25	I	Alive > 567 days
029-15	2.17	48	0	—	—	16	0	Alive > 504 days
030-16	1.88	39	0.1	—	—	16	0	Died on Day 80, cytomegalovirus (CMV) pneumonia
032-18	2.54	27.5	0.2	—	—	24	0	Died on Day 38, hepatitis (non-A, non-B)
033-19	1.29	29.8	1.3	—	—	—	0¶	Died on Day 89, pneumonia, Epstein-Barr virus (EBV) infection§
042-28	—	—		—	—	—	—	Died on Day -1, heart failure, pulmonary edema

*% of cells pre- and post-treatment that form rosettes with sheep rbcs.

†3rd consecutive day of a total wbc count > 1000 cells/mm^3; engraftment confirmed by cytogenetics or rbc phenotyping, if possible, at a later time (*see* text).

‡Seattle criteria.

§Immunoblastic lymphoma found at autopsy in Patient 016-02 (not cause of death) and lymphoproliferative disorder in Patient 033-19 (cause of death).

¶Following the 2nd marrow infusion, ATG and prednisone were given; otherwise, no anti-GvHD medications given prophylactically (*see* text).

TABLE 5.—Clinical outcome for 5 patients with SCID or WA and a mismatched donor

Unique Patient No.	Bone marrow dose ($\times 10^8$ nucleated cells/kg)	E-rosette-positive cells		OKT3 cells		Day of engraftment†	GvHD‡	Outcome
		Pre*	Post	Pre	Post			
023-09	8.08	32	0.1	—	—	—	0	Died on Day 3 post-BMT-2, cardiopulmonary edema
	8.36	23	0	24.3	0	—	—	
026-12	9.83	26	0.1	—	—	20	0	Alive > 525 days
027-13	9.51	22	0	—	—	Not applicable	II	Died on Day 14, infection
028-14	11.31	22	0	—	—	—	II	Alive > 511 days
034-20	4.99	8.1	0.1	—	—	Not applicable	Not applicable	Died on Day 1, pneumonia

* % of cells pre- and post-treatment that form rosettes with sheep rbcs.
†3rd consecutive day of a total wbc count > 1000 cells/mm^3.
‡Seattle criteria.

Only two of the eight fully matched patients developed GvHD and both were only of grade I severity which responded to low-dose prednisone. Except for Patient 033-19 with AA who rejected the first bone marrow allograft and received prednisone and ATG following the second transplant to prevent rejection, none of the other seven patients received any GvHD prophylaxis. Five of the eight matched patients were > 16 years of age. The mean dose of bone marrow given was 2.1×10^8 nucleated cells/kg as shown in table 4.

Two of the eight fully matched patients died from recurrent leukemia on Days 181 and 219 post-BMT. Four other patients died from infections. Two of these four patients developed evidence of immunoblastic lymphomas post-BMT (Patients 016-02 and 033-19), and in one of these patients the lymphoma was proven to be EBV-related. These lymphomas seen in the T-cell-depleted patients are now undergoing characterization.

HLA-Mismatched Recipients

Immunodeficiency

Five patients with immunodeficiency syndromes received allogeneic histoincompatible bone marrow. The degree of identity is detailed in table 2 and the clinical outcome in table 5.

One of these five patients died prior to any signs of engraftment (Patient 027-13) as measured by detectable T lymphocytes in peripheral blood. However, this child did have detectable GvHD at the time of death. A second patient (No. 023-09) who failed to engraft after the first transplant was given ablative therapy prior to the second transplant, died soon afterward, and was not evaluable for graft take or GvHD. A third patient (No. 034-20) died from pneumonia 1 day after his BMT and thus was not evaluable for engraftment or GvHD.

Two of the five patients currently alive have been free of any infections for almost 1 year. The child with WA has not had persistence of his platelet graft but does have T-lymphocyte function in vitro and is phenotypically HLA-identical with his donor by lymphocyte typing and rbc phenotyping.

Malignancy and AA

Twenty-three patients with AA, CML, ALL, AML, and lymphoma received allogeneic histoincompatible bone marrow. The degree of identity is detailed in table 3 and the clinical outcome is shown in table 6.

In the first ten patients of this group (Patients 017-03 to 037-23), only four patients engrafted as defined in this study. One additional patient (No. 020-06) eventually reached a wbc count > 1000/mm^3; however, this patient and one other who is a member of this group of ten patients (No. 024-10) had autologous recovery of bone marrow. These two patients, one of whom had GvHD (Patient 024-10), survived the conditioning therapy yet had no durable graft take. They are described in more detail in another publication in preparation. Both are currently alive, although one of the two has had a leukemic relapse.

All three patients in this initial group of ten patients who received cytarabine in addition to the TBI and cyclophosphamide engrafted well (Patients 017-03, 022-08, and 035-21) as defined by a wbc count > 1000/mm^3 and chromosomal or HLA-phenotype identity with the donor; all three of these patients died. One child with juvenile CML died of recurrent leukemia and one other patient (No. 022-08) died of disseminated aspergillosis. The third patient transplanted at the time of lymphoma relapse (Patient 035-21) died of infection and recurrent tumor.

In this first group of ten patients, not all were evaluable for GvHD because in some there was no marrow take. However, Patient 018-04 had evidence of marrow cells at the time of death and GvHD, although he had not reached the level of engraftment as defined in this study.

TABLE 6.—Clinical outcome for 23 patients with AA, leukemia, or lymphoma and a mismatched donor

Unique Patient No.	Bone marrow dose ($\times 10^8$ nucleated cells/kg)	E-rosette-positive cells		OKT3 cells		Day of engraftment†	GvHD‡	Outcome
		Pre*	Post	Pre	Post			
017-03	5.8	23	0.2	—	—	27	0	Relapsed on Day 48; died on Day 87
018-04	1.09	35.7	0.8	—	—	—	III	Died on Day 43, infection§
020-06	0.99	20.4	0	—	—	—¶	0	Alive > 574 days
	1.68	34	0.6	—	—	—	0	
	3.21	46	0	—	—	—	0	
022-08	2.44	30	0.1	—	—	48	IV	Died on Day 55, aspergillosis
	1.41	27	0	—	—			
024-10	2.02	37	0	—	—	63¶	I	Alive > 539 days; relapsed on Day 243
025-11	2.01	38	0.1	—	—	—	I**	Died on Day 111, fungus§
	2.94	37	0.5	—	—	—	0	
031-17	1.65	26	0.3	—	—	—	Not applicable**	Died on Day 24, infection
035-21	1.42	20	0.3	—	—	24	IV**	Died on Day 53, pneumonia, recurrent tumor
036-22	2.64	25.6	0.1	—	—	—	0**	Died on Day 60, pneumonia
037-23	1.31	30.1	0.9	—	—	—	0**	Died on Day 55, pulmonary edema, infection
038-24	2.11	4	0.3	—	—	21	0††	Died on Day 63, pulmonary edema, renal failure syndrome
039-25	2.27	32	0.1	26	0.16	17	III	Died on Day 73, CMV pneumonia
040-26	—	—	—	—	—	—	—	Died on Day -3
041-27	5.87	23.5	0	16.6	0.6	21	0††	Alive 357 days§
043-29	1.75	27.7	0.1	33.9	0	23	I††	Died on Day 67, DIC, hemorrhagic alveolitis
044-30	1.91	11.2	0.3	20.4	0	20	IV††	Died on Day 58, CMV pneumonia
045-31	5.53	5.1	0	29.7	0.6	19	III	Relapsed on Day 165; died on Day 225
047-32	1.95	16.5	0	15.9	0.3	—	I††	Died on Day 15, bacterial meningitis
048-33	3.09	3.6	0	13.8	0	16	0††	Alive > 266 days
049-34	0.37	34.5	0	33.1	0	—	0††	Died on Day 27, heart failure, liver failure
051-35	1.35	25.0	0	16.8	0.2	15	0††	Relapsed on Day 97; died on Day 121
052-36	2.17	8.6	0	11.8	0	18	0††	Alive > 189 days
054-37	1.14	10.0	0	15.5	0	28	0††	Died on Day 160, CMV encephalitis, Guillain-Barré disease

* % of cells pre- and post-treatment that form rosettes with sheep rbcs.

†3rd consecutive day of a total wbc count > 1000 cells/mm^3.

‡Seattle criteria.

§Patients 018-04 and 025-11 had lymphomatous changes in several organs examined at autopsy but these changes appear unrelated to death; Patient 041-27 developed biopsy-proven histiocytic lymphoma that was treated with acyclovir. There has been no disease recurrence in this patient.

¶Only autologous recovery documented.

** Following infusion of marrow, patients given cyclophosphamide (2.5–7.5 mg/kg every other day) and prednisone (1 mg/kg/day) to prevent GvHD (*see* text).

††Following infusion of marrow, patients given CsA (4 mg/kg/day) and prednisone (1 mg/kg/day) to prevent GvHD (*see* text).

In this group of ten patients, three developed grade II-IV GvHD; in one patient (No. 035-21) this was well-controlled with CsA.

In the second group of 13 patients in tables 3 and 6 (Nos. 038-24 to 054-37), additional chemotherapy was administered along with some anti-GvHD prophylaxis medications as defined earlier (table 6). One patient died prior to BMT from chemotherapy toxicity (Patient 040-26). All of the remaining 12 patients had graft take, but two of the 12 died prior to reaching the level of engraftment as defined in this study (Patients 047-32 and 049-34). Engraftment of donor marrow in the ten patients in this group of 13 occurred between Days 15 and 28 (average, Day 20) and was confirmed by HLA-phenotype and chromosome analysis, when applicable.

Twelve of these 13 patients were evaluable for GvHD (Patient 040-26 died prior to BMT) and only two patients had grade II-IV GvHD (Patients 039-25 and 044-30). All of the other patients had either no signs of acute GvHD (eight patients) or grade I disease (two patients). Some of the patients received CsA and prednisone prophylactically after BMT, as indicated earlier.

Recovery of T-lymphocyte subsets will be reported more completely elsewhere, but in all engrafted patients analyzed thus far, we have documented T-lymphocyte recovery. Two of the mismatched patients who subsequently failed to engraft and who died had evidence of an immunoblastic lymphoma present at autopsy, which may be related to an EBV infection (Patients 018-04 and 025-11).

The incidence of systemic reactions to the pretreated bone marrow was 50% in the recipients of treated marrow. These reactions consisted of urticaria, chills, or fever within 30 minutes after onset of marrow infusion to 60 minutes following completion of infusion. These reactions to the treated bone marrow have been controlled with steroids, diphenhydramine, meperidine, and acetaminophen. Pretreatment of the recipient with these agents prior to the bone marrow infusion has eliminated these reactions and is now performed routinely.

DISCUSSION

The results of the transplant experience on the first 37 patients reconstituted with T-lymphocyte-depleted bone marrow indicate that engraftment can occur with donor marrow following in vitro treatment. All of those patients receiving matched, histocompatible bone marrow for leukemia (seven of seven) had evidence of prompt engraftment, and 13 of the 23 patients with AA or malignancy receiving histoincompatible bone marrow had evidence of engraftment, with two additional patients who only had autologous recovery. The method of T-lymphocyte depletion was reproducible from donor to donor and the technique was internally reproducible when the same donor was used for a second transplant as with Patients 020-06, 025-11, and 033-19. Thus, the in vitro treatment was nontoxic to the graft confirming previous work (28).

All eight patients receiving fully matched HLA-MLC-compatible bone marrow showed no evidence of severe GvHD (grades II-IV). The only patient to receive any anti-GvHD prophylaxis was a child with AA who failed to engraft with her first transplant and was retransplanted and received prednisone and ATG posttransplant. Previously reported data indicate that the incidence of GvHD is 40%-50% (15,29,30) in the fully matched BMT patient with methotrexate, cyclophosphamide, ATG, or steroids following bone marrow infusion. In vitro treatment of the donor bone marrow with OKT3 produced an 18% incidence of GvHD (20). Thus, our results would suggest that no further anti-GvHD prophylaxis is needed for transplants using CT-2 pretreatment of HLA-identical bone marrow. Only three of the eight patients were < 16 years of age, so this population of patients undergoing matched BMT was not skewed to a younger group in which the incidence of severe GvHD is known to be lower.

The administration of methotrexate or other agents to prevent GvHD is not without complications (15). Coccia and co-workers (31) recently reported more rapid engraftment in nine recipients of fully compatible bone marrow not given any methotrexate when compared to five children treated with post-BMT methotrexate. However, five of the nine children who did not receive methotrexate or other anti-GvHD prophylaxis developed severe GvHD. In fact, one of the nine patients received an isograft following which GvHD is highly unusual. Thus, some means of controlling GvHD is necessary, even with HLA-compatible marrow. CT-2 treatment of donor bone marrow appears to be one way to prevent GvHD without the use of methotrexate post-BMT. However, in our study, two of eight matched patients relapsed with their original leukemia, and infections and lymphoma were seen in the others (table 4). The problem of relapse after allogeneic transplantation for ALL has been a major one (32,33), and this study of a small group of matched patients does not yet address the question of relapse or the effect of eliminating GvHD on relapse patterns. However, this question will be addressed in future studies with more long-term survivors.

All of the recipients of mismatched marrow who survived in the study of Powles et al (34) developed severe GvHD, even while receiving CsA post-BMT. Two of our three evaluable patients with immunodeficiency syndromes developed severe GvHD (grades II-IV). However, one of these two patients survived and is now doing well > 1 year post-BMT (Patient 028-14).

Of the first group of ten of our study patients with a malignancy or AA receiving mismatched marrow, three developed GvHD. However, not all ten patients are fully evaluable for GvHD, as indicated earlier, because of a failure of the marrow graft. Thus, the incidence of severe GvHD in this group of ten patients was 50% (2/4). In the

second group of 13 patients, ten had normalization of blood cell counts and were fully evaluable, with an incidence of severe GvHD of 20% (two of ten patients). These rates of severe GvHD indicate that the incidence of GvHD for our patients was comparable to the reported rates for those receiving matched transplants with post-BMT methotrexate as anti-GvHD prophylaxis. This represents an improvement over current approaches to mismatched BMT in this group of patients when compared to one comparable series (34). Because of the high incidence of acute GvHD (50%) in this first group of ten patients, CsA and prednisone were added to reduce this incidence.

The occurrence of GvHD was, however, not the major problem in the mismatched patients. Delayed engraftment or failure of engraftment were the more common problems, and in two of the long-term survivors in this mismatched group, only autologous recovery was documented. Both of these patients gave evidence of autologous lymphocytes in the peripheral blood within the first few weeks post-BMT. This suggests that the ablative therapy for these patients was not sufficient to completely suppress the recipient's residual immune system to allow for the graft survival. Once additional pretransplant immune suppression was added in the form of cytarabine, engraftment in the mismatched patients was less of a problem. This confirmed the observation in the first group of ten mismatched patients for whom satisfactory allograft take was possible in only those patients receiving the additional cytarabine. However, two patients died pretransplant with this additional chemotherapy.

We infer from our experience with T-lymphocyte-depleted marrow that all patients with AA or malignancy who receive histoincompatible bone marrow should receive more intensive ablation prior to transplant to prevent graft rejection and CsA posttransplant (24) to prevent GvHD. The results on the second group of 13 patients (table 6) who received T-lymphocyte-depleted histoincompatible donor marrow suggest that although the intensive immune ablation regimen is very toxic to heavily pretreated patients, engraftment is prompt and no different from graft outcome in patients receiving fully compatible transplants. Failure to graft was only seen in those patients not given cytarabine in addition to cyclophosphamide.

Two of the objectives of this study have already been met: (*a*) BMT has been extended to those without fully compatible donors by using T-lymphocyte depletion of the donor marrow, and (*b*) GvHD has been reduced in incidence and severity in the mismatched transplants and virtually eliminated in the matched transplants. T-lymphocyte depletion was found to be effective and highly reproducible as well as nontoxic to the graft. However, toxicity to the patient due to the additional ablative therapy required for graft take, the problem of relapse even with this additional therapy, and the risk of subsequent infections and lymphoma development are all formidable problems that must be weighed against the use of techniques to deplete marrow of T lymphocytes.

The third objective remains to determine the effect of GvHD on survival and relapse. Two of the matched patients have experienced relapses of their leukemia and most of the surviving mismatched patients with malignancy require additional follow-up to make any assessment of relapse. A more definitive answer on the important issues of tumor relapse, secondary tumor development, and ultimate immunological recovery must await further study. Our experience to date suggests that CT-2 ablation of cells with GvHD capability can reduce GvHD in the matched patient without the need for anti-GvHD medication and permit graft take in the histoincompatible situation with a relatively low incidence of severe GvHD.

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Randomized Trial Comparing Vindesine Plus Cisplatin With Vinblastine Plus Cisplatin in Patients With Non-Small Cell Lung Cancer, With an Analysis of Methods of Response Assessment[1,2,3]

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One hundred eight patients with stage III non-small cell lung cancer were randomly assigned to receive cisplatin (120 mg/m^2) with either vindesine (3 mg/m^2) or vinblastine (6 mg/m^2). None had previously received chemotherapy. An additional goal was to determine if the observation of visible evaluable lesions was as accurate a method of response assessment as the observation of bidimensionally measurable lesions in non-small cell lung cancer. When vindesine plus cisplatin was compared to vinblastine plus cisplatin, response rates (33% vs 41%), median response durations (8.6 vs 5.6 months), and median survival times of responding patients (18.4 vs 16.2 months) were similar. Response rates, response durations, and survival times of responding patients determined through the observation of evaluable or measurable indicator lesions did not differ significantly. More patients receiving vinblastine plus cisplatin experienced wbc counts < 2100/mm^3 (P = 0.003). The two regimens demonstrated comparable response and survival data but clinically significant leukopenia was more common in vinblastine-treated patients. There was no difference in response data obtained through the study of patients with measurable and evaluable indicator lesions. [Cancer Treat Rep 69:387–395, 1985]

Because of limited success using conventional antineoplastic drugs, several centers have investigated new single agents in patients with non-small cell lung cancer (NSCLC). The vinca alkaloid vindesine was identified at six institutions as having an overall activity of 23% in 190 previously untreated patients (1–6). In addition, vindesine alone proved superior to a combination of the conventional agents cyclophosphamide plus doxorubicin in a randomized trial (7).

Cisplatin in phase II trials has been demonstrated to have major antitumor activity in 20% of 142 previously untreated patients wth NSCLC (8–13). The differing side effects of vindesine and cisplatin enabled the two drugs to be used at the same therapeutic dose studied in phase II trials in a combination, producing a 43% major response rate and a 22-month median survival for responding patients randomized to receive vindesine plus cisplatin at 120 mg/m^2 (14). Results of trials at other institutions have confirmed the activity of this combination (3,15–18).

Studies performed with vinblastine in the 1960s did not identify it as an active agent against NSCLC. After activity was observed with vindesine, trials with vinblastine were reinitiated. Indications of antitumor efficacy were seen in a phase I trial that included patients with NSCLC (19). Schulman et al (20) conducted a formal phase II study of vinblastine that yielded a 27% major response rate in 22 patients, with a median response duration of 5.5 months. Trials combining vinblastine given on a weekly bolus schedule with cisplatin have produced re-

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sponse rates similar to those achieved with vindesine plus cisplatin and have shown manageable toxicity (21-23).

Since both vindesine plus cisplatin and vinblastine plus cisplatin have demonstrated good response rates and acceptable toxicity, this present study was undertaken to compare the two regimens using a randomized design to determine whether there are differences in response rate, response duration, survival, and toxicity (particularly leukopenia and peripheral neuropathy).

An additional objective of the current trial was to determine whether the observation of clinically apparent evaluable lesions is as accurate a method of response assessment as the observation of bidimensionally measurable lesions in patients with NSCLC. In 1979, Eagan et al (24) reported the feasibility of using visible but not easily measurable indicator lesions to assess response in lung cancer trials. In their study, when the response rates, times to response, response durations, or survival times of chemotherapy-treated patients with bidimensionally measurable and nonmeasurable lesions were compared, no significant differences were detected. This aspect of the trial was conducted to determine whether similar results could be found using a different treatment regimen.

PATIENTS AND METHODS

From September 1981 to July 1982, 108 patients with histologically confirmed epidermoid carcinoma, adenocarcinoma, or large cell carcinoma of the lung were entered in the study. All patients had clinical stage III disease as established by the criteria of the American Joint Committee for Staging (25). Patients were required to have either bidimensionally measurable indicator lesions or visible evaluable lesions (defined below) on physical examination, chest roentgenogram, or computed tomographic (CT) scan. Eligibility requirements also included: age < 70 years old; creatinine clearance > 64 ml/minute; leukocyte count > 3900/mm^3; platelet count > 100,000/mm^3; Karnofsky performance status > 50%; no clinical congestive heart failure; and a stable heart rhythm. Only patients who had not previously received chemotherapy were eligible for this trial. Informed consent was obtained from all patients.

The types of measurable and evaluable lesions used to determine response are shown in table 1. Patients with measurable disease had lesions with clearly defined perpendicular diameters seen on physical examination, chest roentgenogram, or CT scan. Evaluable lesions were clinically apparent but not bidimensionally measurable and consisted of pulmonary infiltrates on chest roentgenogram in 86% of this group of patients. Lesions seen on thoracic or abdominal CT scan, pleural effusions, or a matted lymph node mass on physical examination were also included. Hepatomegaly on physical examination, previously irradiated lesions, or abnormalities seen only on radionuclide scans were specifically excluded as measurable or evaluable lesions.

TABLE 1.—Types of indicator lesions used to assess response

Indicator lesion	%
Measurable-disease patients (n = 53)	
Lung mass on chest x-ray	64
Lymph node on physical examination	19
Subcutaneous nodule on physical examination	13
Lung mass on CT scan	4
Evaluable-disease patients (n = 44)	
Pulmonary infiltrate on chest x-ray	86
Pleural effusion on chest x-ray (cytology-positive)	5
Pulmonary infiltrate on CT scan	5
Lymph node on physical examination	2
Liver metastasis on CT scan	2

Before randomization, all patients had a complete medical history, physical examination, and laboratory evaluation including cbc, 12-channel biochemical profile, serum electrolytes, prothrombin time, 12- or 24-hour urine collection for creatinine, and chest roentgenogram. Radionuclide scans were performed only when clinically indicated. Follow-up studies included weekly histories, physical examinations, and cbcs for the next 6 weeks of treatment. Thereafter, these studies were repeated biweekly. Biochemical tests, serum electrolytes, creatinine clearance, chest roentgenograms, and CT scans of indicator lesions were repeated before each cisplatin dose or at least every 6 weeks.

Prior to randomization, patients were stratified according to the method of response assessment employed (measurable or evaluable indicator lesions) and performance status (80%-90% or 60%-70%, Karnofsky scale). Randomization was done by the selection of sealed cards.

All patients received cisplatin at a dose of 120 mg/m^2 of body surface area. Hydration with 2-3 L of 5% dextrose in 0.45% NaCl was initiated 12-18 hours before therapy. Immediately before cisplatin, mannitol (12.5 g) was given as a rapid iv injection. Cisplatin was then administered over 20 minutes. For the next 6 hours patients received an iv infusion of 20% mannitol at a rate of 50 ml/hour and 5% dextrose in 0.45% NaCl at 200 ml/hour. After the first 6 hours, the total fluid output was replaced with an equal volume of 5% dextrose in 0.45% NaCl for at least 18 hours (26). Cisplatin was administered on Days 1 and 29 and then every 6 weeks. All cisplatin was given in the hospital. High doses of iv metoclopramide with or without concomitant dexamethasone and diphenhydramine were utilized as an antiemetic in 90% of patients (27,28); 10% received delta-9-tetrahydrocannabinol as part of a randomized study comparing it to metoclopramide (29).

Patients were randomly assigned to receive either vindesine (3 mg/m^2) or vinblastine (6 mg/m^2) (supplied by Eli Lilly and Co, Indianapolis, IN). Both drugs were given as rapid iv injections on an identical schedule: weekly for 5

weeks (Days 1, 8, 15, 22, and 29), then every other week. Chemotherapy was continued until there was evidence of progressive disease or unacceptable toxicity.

Subsequent chemotherapy was given at the same doses if the leukocyte count at that time was > 3000/mm^3 and the platelet count was > 100,000/mm^3. The vindesine or vinblastine dosages were reduced by 50% if the leukocyte count was between 2000 and 3000/mm^3 or if the platelet count was between 75,000 and 100,000/mm^3. Vindesine, vinblastine, and cisplatin were withheld if the leukocyte or platelet counts were lower than these levels. Vindesine and vinblastine were withheld if peripheral neuropathy was severe and were decreased by 50% if symptoms were moderate (interfering with normal activities). Cisplatin was decreased by 50% if the creatinine clearance fell to 45–55 ml/minute and was withheld if the creatinine clearance was ⩽ 44 ml/minute. Adequate treatment was defined as one course of therapy, including a minimum of one dose of cisplatin and three doses of either vindesine or vinblastine. Assessment of response was made with each repeat physical examination, chest roentgenogram, or CT scan.

Therapeutic responses for patients with measurable disease were defined as follows. Complete remission was defined as the disappearance of all evidence of tumor for at least 4 weeks. Partial remission was defined as a > 50% reduction of the sum of the product of the two greatest perpendicular diameters of all indicator lesions for at least 4 weeks. Minor response was defined as a > 25% reduction of all indicator lesions, but less than that required for a partial remission. Stable disease was defined as a < 25% change in the size of all indicator lesions for a minimum of 90 days. Disease progression was defined as the appearance of new lesions or an increase in indicator lesions by > 25%.

Response categories for patients with evaluable disease were complete remission, improvement, stable disease, and disease progression. Improvement was defined as a definite decrease in the size of the indicator lesion(s) noted by three investigators, including the reference radiologist; it is considered a major response category. The definition of complete remission was the same for patients with evaluable and measurable disease. Stable disease was defined as no objective change in any disease parameter for a minimum of 90 days. Disease progression was defined as unequivocal worsening of any evaluable indicator lesion as determined by three investigators or the appearance of new lesions. Survival and duration of response were recorded from the first day of treatment.

The statistical significance of observed differences in the percentage of patients who responded was assessed by the chi-square test, or when appropriate, the Fisher's exact test. The product-limit method (30) was used to estimate the distribution of response duration and survival time for subgroups of patients. Tests for differences in response duration and survival time were done using the log-rank test (31). Differences yielding observed significance levels > 5% were not considered statistically significant.

RESULTS

Ninety-seven of the 108 patients entered in this study were considered adequate for both response assessment and toxicity. Eight of the 11 patients not evaluated for response received inadequate treatment (fewer than three doses of vindesine or vinblastine) because of early death (three patients) or poor functional status (five); two were lost to follow-up and one developed a pneumothorax that made further assessment of the indicator lesion impossible. The two treatment groups were well-matched for age, sex ratio, performance status, histologic type of lung cancer, and prior surgery and/or irradiation (table 2). Sites of metastasis, numbers of metastatic sites, and extent of disease were also similar (table 2), except for an increased number of patients with liver metastases in the vindesine plus cisplatin arm (15% vs 2%) ($P = 0.0249$). Vindesine plus cisplatin patients received a median of three doses of cisplatin and ten doses of vindesine; vinblastine plus cisplatin patients received a median of two doses of cisplatin and eight doses of vinblastine. Median follow-up is 18 months (range, 13–23+).

Table 3 shows therapeutic results. Major response rates were similar for patients treated with vindesine plus cisplatin (33%) and vinblastine plus cisplatin (41%) ($P = 0.446$). There was a significant difference ($P = 0.006$) in the major response rate when all patients with a performance status of 80% and 90% were compared to all patients with a performance status of 60% and 70% (50% vs 21%). Among the patients with a performance status of 80% and 90% or 60% and 70%, there was no difference in response rate when patients who received vindesine plus cisplatin were compared to those who received vinblastine plus cisplatin. When the major response rates determined by the observation of measurable lesions and evaluable lesions were compared, the data from patients with evaluable disease did not differ significantly from those from patients with measurable disease (41% vs 34%; $P = 0.0481$) (table 4). Response rates among the measurable- and evaluable-disease patients did not differ when the two treatment regimens were compared. There was no significant difference ($P = 0.386$) in response rates among patients with adenocarcinoma (36%), epidermoid carcinoma (46%), or large cell carcinoma (17%), regardless of the treatment regimen employed. Response rates did not differ significantly between patients with and without prior radiotherapy (38% vs 37%).

As shown in figure 1, there was no significant difference in the duration of response comparing patients receiving vindesine plus cisplatin to those treated with vin-

TABLE 2.—Characteristics of 97 adequately treated patients*

	Total (n = 97)	Vindesine + cisplatin (n = 48)	Vinblastine + cisplatin (n = 49)
Median age in yrs (range)	55 (29-72)	56 (29-69)	54 (35-72)
Sex			
Male	67	60	74
Female	33	40	26
Performance status (Karnofsky)			
60% + 70%	44	44	45
80% + 90%	56	56	55
Histologic cell type			
Adenocarcinoma	69	61	78
Epidermoid carcinoma	25	29	20
Large cell carcinoma	6	10	2
Prior therapy			
None	56	53	59
Surgery	6	8	4
Radiation therapy	28	29	27
Surgery + radiation therapy	10	10	10
Sites of metastasis			
Lymph nodes	62	58	65
Lung	40	38	43
Bone	23	23	22
Pleura	20	21	19
Brain	19	19	19
Subcutaneous	11	13	10
Liver	8	15	2
Other	10	10	10
No. of metastatic sites			
1	46	48	45
2	27	23	31
⩾ 3	27	29	24
Stage			
III M0	14	10	16
III M1	86	90	84

* Unless otherwise specified, values = % of patients.

blastine plus cisplatin (8.6 vs 5.6 months; P = 0.444). Response durations were significantly longer in patients with a performance status of 80% and 90% (8.6 months) compared to patients with a performance status of 60% and 70% (4.4 months) (P = 0.007). These differences in the duration of response by performance status were not affected by the drug regimen used. There was no significant difference in the duration of response determined through the observation of measurable or evaluable indicator lesions (fig 2), regardless of the chemotherapy ad-

TABLE 3.—Therapeutic responses of patients receiving vindesine + cisplatin or vinblastine + cisplatin*

Response	Total (n = 97)	Vindesine + cisplatin (n = 48)	Vinblastine + cisplatin (n = 49)
Major response	36 (37)	16 (33)	20 (41)
Complete remission	5 (5)	2 (4)	3 (6)
Partial remission†	13 (13)	6 (13)	7 (14)
Improvement‡	18 (19)	8 (16)	10 (21)
Minor response†	6 (6)	2 (4)	4 (8)
Stable disease	25 (26)	14 (29)	11 (23)
Disease progression	30 (31)	16 (34)	14 (28)

* Values = No. of patients (%).
†Patients with measurable disease only.
‡Patients with evaluable disease only.

TABLE 4.—Comparison of response data obtained from the study of patients with measurable and evaluable lesions of NSCLC

	Patients with measurable lesions	Patients with evaluable lesions	*P* value
Major response rate*	34%	41%	0.481
Median remission duration (mos)	7.8	9.8	0.438
Median survival of responding patients (mos)	17.5	18.4	0.689

* Complete + partial response in patients with measurable lesions and complete response + improvement in patients with evaluable lesions.

ministered. Response durations did not differ significantly for all patients and for each treatment arm when the histologic cell types of NSCLC were examined.

Survival measured from the start of therapy did not differ significantly when the patients responding to vindesine plus cisplatin (18.4 months) were compared to the patients responding to vinblastine plus cisplatin (16.2 months), as shown in figure 1. The median survival for all patients in the study was 9.5 months (vindesine arm, 8.5 months; vinblastine arm, 12.3 months; $P = 0.176$). Survival differences were noted in responding patients with a higher performance status (80% and 90%) when compared to responding patients with a lower performance status (60% and 70%) (20.7 vs 12.3 months; $P = 0.006$). There was no difference in survival between responding patients with measurable indicator lesions and evaluable indicator lesions when all responding patients or when the two regimens were compared (fig 2). Significant differences were not observed when the survival of patients with epidermoid carcinoma was compared to the survival of patients with adenocarcinoma, regardless of the treatment regimen employed.

One hundred six of the 108 randomized patients were evaluated for toxicity. The two patients excluded were

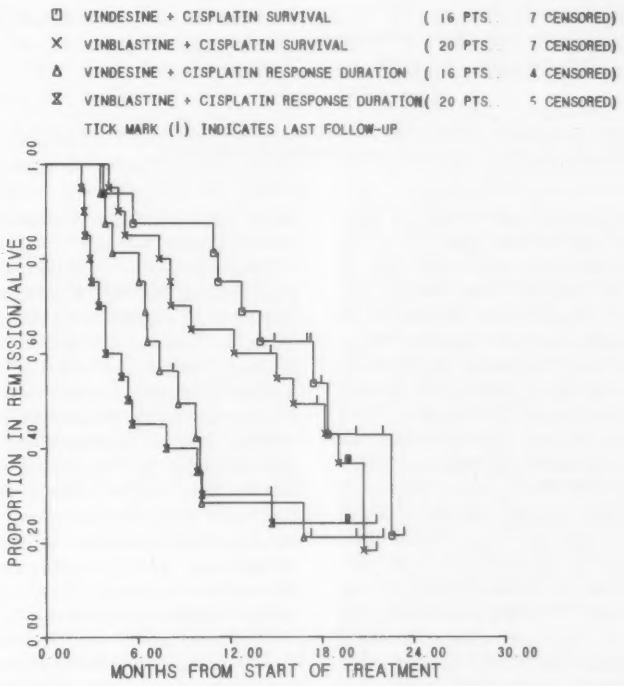

FIGURE 1.—Survival (upper 2 curves) and duration of response (lower curves) for patients treated with vindesine + cisplatin or vinblastine + cisplatin achieving major response (complete or partial remission in measurable-disease patients; improvement in evaluable-disease patients).

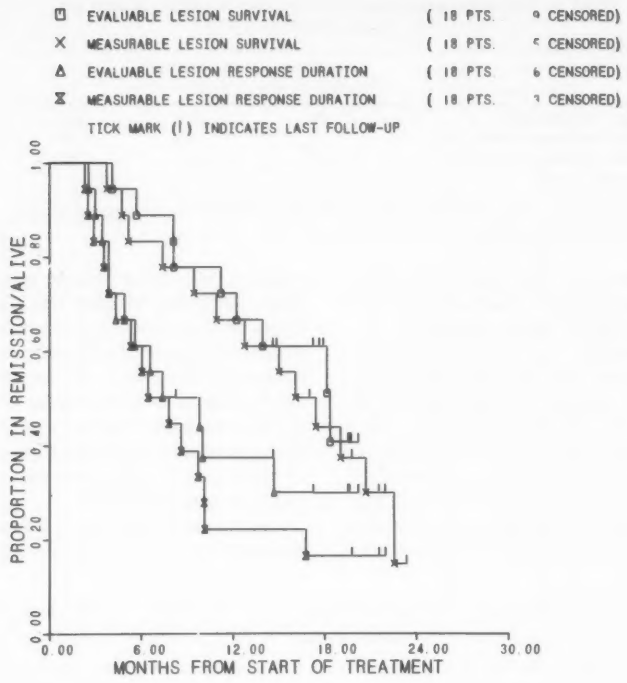

FIGURE 2.—Remission duration (lower 2 curves) and survival (upper 2 curves) comparing all responding patients with measurable and evaluable indicator lesions.

lost to follow-up after the initial doses of therapy were given. Table 5 shows the toxic effects observed during the study. The median leukocyte count nadir for all patients was 2400/mm^3; it was 3000/mm^3 for patients treated with vindesine plus cisplatin and 2000/mm^3 for patients treated with vinblastine plus cisplatin. When the two treatment arms were compared, significantly more patients who received vinblastine plus cisplatin experienced leukocyte count nadirs < 2100/mm^3 (53% vs 27%; $P = 0.003$) and more frequent hospitalizations for fever occurring during a period of drug-induced leukopenia (19% vs 8%; $P = 0.05$). Two patients died with fever during a period of drug-induced leukopenia, one in each treatment arm. The leukocyte count nadirs were seen 2 weeks after the start of treatment for both regimens. Dose attenuation due to thrombocytopenia was never necessary on either arm. No study patient required a platelet transfusion or developed clinical bleeding related to thrombocytopenia. The median platelet count nadir for vindesine-treated patients was 177,000/mm^3; it was 176,000/mm^3 for those treated with vinblastine. Four patients on the vindesine plus cisplatin arm and two on the vinblastine plus cisplatin arm developed platelet counts < 75,000/mm^3 at any time during their treatment. The median lowest recorded hemoglobin was 9.2 g for both treatment arms.

Forty-nine percent of patients who received vindesine plus cisplatin and 38% of those who received vinblastine plus cisplatin experienced a rise in creatinine > 1.4 mg/dl (table 5). One patient died during an episode of nonoliguric renal failure and another required temporary dialysis. Four patients had cisplatin discontinued because of persistent serum creatinine elevation.

Some degree of peripheral neuropathy occurred in all patients (table 5). The effects were generally mild (decreased deep tendon reflexes or paresthesias) in both treatment arms, although symptoms appeared more rapidly in patients receiving vindesine. Moderate symptoms of weakness, activity-limiting paresthesias, or paralytic ileus occurred in equal numbers of patients (7%) who received vindesine plus cisplatin or vinblastine plus cisplatin. Moderate toxicity occurred earlier in the course (2–12 weeks) of vindesine-treated patients than of vinblastine-treated patients (> 12 weeks). No patient had treatment discontinued because of neurotoxicity.

Severe nausea and vomiting occurred in < 20% of patients and with equal frequency on both treatment arms. Most patients received high doses of iv metoclopramide

TABLE 5.—Toxicity from vindesine + cisplatin and vinblastine + cisplatin therapy*

	Total (n = 106)	Vindesine + cisplatin (n = 53)	Vinblastine + cisplatin (n = 53)
Leukocyte count nadir (cells/mm^3)			
> 4000	17 (16)	11 (21)	6 (11)
3000-3900	23 (22)	17 (32)	6 (11)
2100-2900	24 (23)	11 (21)	13 (25)
1000-2000	27 (25)	10 (19)	17 (32)
< 1000	15 (14)	4 (8) (P = 0.03)	11 (21)
Platelet count nadir (cells/mm^3)			
> 100,000	90 (85)	44 (83)	46 (87)
75,000-99,000	10 (9)	5 (9)	5 (9)
< 75,000	6 (6)	4 (8)	2 (4)
Maximum serum creatinine (mg/dl)			
≤ 1.4	60 (56)	27 (51)	33 (62)
1.5-2.0	26 (25)	13 (25)	13 (25)
2.1-3.4	17 (16)	11 (21)	6 (11)
≥ 3.5	3 (3)	2 (4)	1 (2)
Degree of peripheral neuropathy			
None	0 (0)	0 (0)	0 (0)
Mild	98 (93)	49 (93)	49 (93)
Moderate (interferes with function)	8 (7)	4 (7)	4 (7)

* Values = No. of patients (%).

or metoclopramide plus dexamethasone as an antiemetic (27,28). Eight patients (six who received vindesine plus cisplatin and two who received vinblastine plus cisplatin) developed clinically apparent hearing loss. Four of the eight patients had previously received whole-brain irradiation. Therapy was discontinued in two patients because of ototoxicity. Both patients had received prior whole-brain irradiation. Patients who received whole-brain radiotherapy prior to receiving chemotherapy experienced more clinically apparent hearing loss (21%) than those who did not (5%) (P = 0.038). Forty-six percent of the patients who were given vindesine plus cisplatin experienced complete or near complete alopecia as compared to 31% of those who were given vinblastine plus cisplatin. Constipation was mild, occurred with equal frequency in both treatment arms, and responded well to laxatives. Cisplatin therapy was discontinued in two vinblastine-treated patients because of hypersensitivity reactions immediately following cisplatin administration. Symptoms developed during the second and sixth courses of cisplatin.

DISCUSSION

Combinations of individually active antineoplastic agents given at therapeutic dosage can produce improved response rates, response durations, and survival times in patients with NSCLC when compared to the individual drugs used alone. The response data from this trial are in accord with those from previously reported trials investigating combinations of vinca alkaloids and cisplatin (14-18,21-23). Furthermore, this study confirms the previously published observations documenting the overall activity of cisplatin-containing regimens in this disease (32,33).

Response rates, response durations, and survival times did not differ significantly when patients treated with vindesine plus cisplatin and vinblastine plus cisplatin were compared. Significant differences in response rates, response durations, and survival times were observed when all responding patients with a performance status of 80% and 90% were compared to all responding patients with a performance status of 60% and 70%, regardless of the treatment regimen employed.

Leukopenia was a major dose-limiting toxic effect. Significantly more patients on the vinblastine plus cisplatin arm experienced leukocyte count nadirs < 1000/mm^3 and were hospitalized for fever during a period of drug-induced leukopenia. Changes in vinblastine plus cisplatin dosages or schedules may alter the myelosuppression observed; however, response rates may also be affected. Since less leukopenia was observed with the vindesine plus cisplatin combination, this regimen may be more suitable for trials which add another active single agent to the vinca alkaloid plus cisplatin combination, particularly when myelosuppression is a side effect of the third drug.

Although paresthesias occurred more quickly in vindesine plus cisplatin patients, there was no difference between vindesine- and vinblastine-treated patients when the number of patients with neuropathy that interfered with function was compared. Alopecia was less frequent and of a lesser degree in vinblastine plus cisplatin-treated

patients. The incidence of thrombocytopenia, anemia, nephrotoxicity, ototoxicity, nausea, emesis, constipation, and cisplatin hypersensitivity was similar for patients on either treatment regimen. As noted previously (34), moderate ototoxicity occurred more often in patients who had received prior whole-brain irradiation. Severe, treatment-limiting hearing loss was seen only in these patients.

The response rates, response durations, and survival times obtained through the study of patients with evaluable lesions were similar to those obtained by the assessment of response in patients with bidimensionally measurable lesions of NSCLC. Our findings confirm the earlier observations of Eagan et al (24), who concluded that response criteria used for patients with evaluable, nonmeasurable NSCLC were valid in assessing response to therapy.

Both vindesine plus cisplatin and vinblastine plus cisplatin are active regimens in patients with NSCLC and either can be used safely in the treatment of this illness. Other regimens combining a plant alkaloid (etoposide) with cisplatin have also yielded good response rates and improved survival times in responding patients (35,36). In all of these trials, however, most patients do not respond. While the results of the current studies with plant alkaloids and cisplatin are encouraging, further studies are needed to identify new agents, to establish new combinations of individually active agents, and to define the roles of radiotherapy and surgery in chemotherapy-treated patients.

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Clinical-Biologic Patterns of Metastatic Melanoma and Their Effect on Treatment[1,2]

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We have identified three distinct groups of patients with metastatic malignant melanoma that differ in their clinical behavior and responsiveness to therapy. These include a favorable group of patients with lymph node or skin metastases, an intermediate prognostic group with lung or bone metastases, and a poor prognostic group with brain or gastrointestinal involvement. These three groups differed in their predicted objective response to therapy, in their likelihood to achieve a complete remission, and in their median survival.
[Cancer Treat Rep 69:397–401, 1985]

Clinically, it has been evident for some time that metastatic malignant melanoma may present with widespread metastases, including skin, subcutaneous lesions, lymph nodes, lung, liver, gastrointestinal tract, adrenal, heart, kidney, central nervous system, thyroid, and spleen in an apparently random fashion (1–15). Stehlin et al (1), Einhorn et al (2), and Amer et al (3) have noted various clinical patterns of metastases and differences in response to chemotherapy and survival dependent on these patterns. However, prognostic factors predicting the likelihood of developing metastases and survival after such metastases have not been well-characterized.

Recently, it has been published that murine melanoma tumors may be heterogeneous and contain subpopulations of cells with diverse biologic behavior (16–19). These tumors may exhibit predominantly pulmonary metastases and various extrapulmonic metastases including heart, brain, skin, or lymph node. Microenvironment, vascular anatomy, and intrinsic properties within the heterogeneous tumor cells are hypothesized to alter the site of selection of metastases.

We present our classification of metastatic malignant melanoma based on clinical metastatic patterns and discuss prognostic factors, response to therapy, and survival of patients with these clinical patterns. We hypothesize that human malignant melanoma has defined biologic subtypes which can be identified as metastatic patterns. Possible biologic parameters underlying the clinical patterns are also discussed.

MATERIALS AND METHODS

During the period from January 1, 1973, to December 7, 1983, 490 patients with malignant melanoma were followed at the University of Arizona Cancer Center, of whom 216 developed metastatic disease. Records were compiled for the clinical history from the date of original diagnosis to the last date of follow-up or death. The date of initial diagnosis was the date of histologically proven malignant melanoma. All patients had histologic confirmation of primary and metastatic disease. Median time to last follow-up was 28 months from the date of initial diagnosis of the 216 patients who eventually developed metastases. One hundred seventy-five of 216 patients with metastases have died. The date of first metastasis was the date of histologically proven metastatic disease. Staging criteria were as described by the International Union Against Cancer classification.

Criteria used for clinical response were as follows: *complete response (CR)*—the disappearance of all evidence of tumor for at least 4 weeks; *partial response (PR)*—a > 50% reduction in the sum of the products of the two greatest perpendicular diameters of all measurable lesions for at least 4 weeks; and *no response*—a < 50% response, mixed response, stable disease, or disease progression.

The Kaplan and Meier methods (20) for statistical estimation of the survival-type curves reflecting the time from diagnosis of metastatic disease to the last date of

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contact or death were used. Methods used to compare survival-type curves included the generalized Wilcoxon method of Gehan (21), the log-rank method of Mantel (22), and the generalized Kruskal-Wallis method of Breslow (23). Multivariate analysis of metastatic sites used the proportional hazard model introduced by Cox (24).

RESULTS

Survival of Patients With Single Metastasis Versus Multiple Metastases at Initial Presentation

Two hundred sixteen of 490 patients (44%) with malignant melanoma developed metastatic (stage III) disease. Of these 216 patients with metastatic melanoma, 124 patients (57%) had only a single organ involved at the initial detection of their first metastasis, whereas 92 patients (43%) had multiple (two or more) organs involved. The median survival for patients with a single organ metastasis was 12 months compared to 8 months for those patients with multiple organs involved (table 1). This difference was highly significant ($P = 0.004$).

Survival With Single Metastasis

Analysis of survival by organ site for those patients with only a single metastasis revealed that those patients with skin or lymph node metastases survived significantly longer than those with visceral disease ($P < 0.001$; Breslow). Patients with only lung metastases had an intermediate survival pattern since a significant proportion of these patients did well initially but later failed rapidly and approached the survival of the poorer risk patients with gastrointestinal (GI) tract, bone, or brain metastases (table 1).

For example, the 75th percentile for patients with lung metastases was similar to the good prognostic group of patients with skin or nodal metastases; the 50th percentile for patients with lung metastases exhibited an intermediate survival; however, the 25th percentile for patients with lung metastases was quite similar to the poor prognostic group of patients with GI tract or brain involvement.

Metastatic Involvement Versus Noninvolvement by Organ Site

Once a patient with melanoma develops any metastasis the prognosis is poor, with a < 5% survival rate at 4 years. However, involvement of particular sites of metastases predicted poorer survival (table 2). Poorer survival was predicted by a metastasis to bone ($P = 0.04$), lung ($P = 0.01$), GI tract ($P < 0.001$), or brain ($P < 0.001$).

Poorer Survival Predicted by Multivariate Analysis of Metastatic Sites

To further substantiate or refute that the six sites of metastatic organ involvement might have prognostic importance, a Cox multivariate regression analysis was performed (table 3). Visceral sites of metastases predicted a much poorer outcome and were highly significant. A poorer survival was predicted by GI tract ($P < 0.0001$), brain ($P = 0.001$), lung ($P = 0.005$), or bone ($P = 0.01$) involvement. Once a metastasis had occurred, skin or lymph node involvement did not further help predict a poorer survival since it was those patients who had predominantly visceral disease who did poorly, whether or not they also had skin or nodal involvement concurrently. The presence of skin or lymph node metastases, whether as a single site or concurrently with visceral disease, did not yield additional prognostic information.

Objective Response by Metastatic Site

The response to therapy was analyzed according to the site of initial metastatic organ involvement (table 4). Patients with a single metastasis responded with equal frequency by site of metastasis when compared to patients with multiple metastases. However, patients with a single metastasis were more likely to achieve CR, whereas those patients with multiple metastases usually ob-

TABLE 1.—Survival of patients with single metastasis vs multiple metastases at initial presentation

Pattern	No. of patients	Survival in mos by percentile			
		75th	50th	25th	
Multiple metastases	92	3	8	17	$P = 0.04$*
Single metastasis	124	7	12	23	
Skin	42	9	17	32	
Node	33	9	13	23	
Lung	16	7	8	13	
GI tract	13	2	8	16	$P < 0.001$†
Bone	10	3	6	9	
Brain	10	2	5	12	

* Survival of patients with single metastasis vs multiple metastases; $P = 0.004$ (log-rank).
†Survival of patients with single metastasis by organ involvement; $P < 0.001$ (Breslow).

TABLE 2.—Poorer survival predicted by site of metastatic involvement

Site	No. of patients*	Median survival (mos)	Significance if involved (yes vs no)†
Skin			
Yes	94	11	NS
No	122	11	
Node			
Yes	90	11	NS
No	126	11	
Lung			
Yes	64	8	$P = 0.01$
No	152	12	
Bone			
Yes	15	7	$P = 0.04$
No	201	11	
GI tract			
Yes	56	4	$P < 0.01$
No	100	12	
Brain			
Yes	32	4	$P < 0.001$
No	184	12	

* Patient may have had ⩾ 1 metastatic site.
† Log-rank, 2-tailed. NS = not significant.

tained no more than a PR. Patients with CR lived significantly longer than did nonresponders (median, 27 vs 9 mos; $P < 0.001$; log-rank). PR did not significantly alter survival (median, 12 vs 9 mos; $P > 0.10$; log-rank).

Patients with skin and lymph node metastases had a higher response rate and accounted for 12 of 15 of those patients who achieved CR. They also had the longest median survival (11 months). Patients with pulmonary metastases had an intermediate median survival of 8 months and an intermediate objective response rate of 16%, with three of nine responders (33%) achieving CR. Patients with bone, GI tract, or brain metastases responded objectively only 13% of the time. Twelve of the 13 responding patients (92%) had a short-lived PR that did not have a significant survival benefit. The median survival for patients with bone metastases was 7 months and quite similar to the 8 months noted in patients with pulmonary metastases. The median survival for patients with brain or GI tract metastases was poor and was only 4 months.

TABLE 3.—Multivariate analysis predicting poorer survival by site of initial metastasis

Site*	Significance†
GI tract	$P < 0.001$
Brain	$P = 0.001$
Lung	$P = 0.005$
Bone	$P = 0.01$
Skin	NS
Node	NS

* Patient may have had ⩾ 1 metastatic site.
† Cox regression model. NS = not significant ($P > 0.05$).

DISCUSSION

Univariate analysis, multivariate analysis, and response to therapy support the existence of three groups of patients with metastatic melanoma. (*a*) Patients with skin or nodal metastases had the best prognosis, with a median survival of 11 months and a higher objective response rate of 24%; they were the most likely to achieve a CR. (*b*) Patients with bone and lung metastases had an intermediate prognosis, with a median survival of 7 and 8 months, respectively, and an intermediate response rate of 14% and 16%, respectively; one-third of responders with lung metastases achieved CR. (*c*) Patients with brain or GI tract involvement had poor prognosis, with a median survival of 4 months and a low objective response rate; those who responded only had short-lived PR. Patients who achieved CR lived significantly longer ($P < 0.001$; log-rank), whereas patients who obtained a PR or less did not show any survival benefit.

Patients with boney metastases were difficult to classify because of their small number and overlapping characteristics with other groups, a result also noted by others (4,9). For example, the patients predicted a similar outcome as did the patients with lung metastases (intermediate group) when analyzed by site of involvement, by multivariate analysis, and by median survival (tables 2–4, respectively). On the other hand, when only a single metastasis was present (table 1), patients with boney metastases did act similar to the poorer prognostic group of patients with brain or GI tract metastases with regard to their poor survival. Although patients with boney metastases had a similar response rate of 14% compared to the

TABLE 4.—Response and survival by initial site of organ metastasis*

Prognosis	Site	No. of patients†	Median survival (mos)	Response (%)‡		
				CR	PR	Objective
Better	Skin	94	11	10	15	25 (27)
	Node	90	11	8	10	18 (20)
Intermediate	Lung	64	8	3	6	9 (16)
	Bone	15	7	0	2	2 (14)
Poorer	GI tract	56	4	1	5	6 (11)
	Brain	32	4	0	5	5 (16)
Total		211	9	15 (7)	24 (11)	39 (18)

* Patients with other metastatic sites were excluded from this analysis (2, breast; 2, bladder; and 1, spleen).

† Patient may have had ⩾ 1 metastatic site.

‡ CR vs no response: $P < 0.001$, log-rank. PR vs no response: $P > 0.10$, log-rank. Survival of all responders (median, 17 mos) vs nonresponders (median, 9 mos): $P = 0.005$, log-rank.

16% response rate of patients with lung metastases, they did not achieve any CRs. The absence of CRs in patients with boney metastases may be due in part to the small sample size. On the other hand, CRs may be more difficult to obtain since patients with boney metastases usually have multiple distant sites involved even though a single system is involved. Patients with boney metastases were assigned to the intermediate prognostic group since they had many similarities and few dissimilarities to the patients with lung metastases.

In our study, patients with metastatic melanoma with metastases to a single organ had a significantly prolonged survival, particularly if the skin or lymph nodes were involved, compared to patients with visceral disease, who had a universally poor prognosis. This is a result comparable to that reported by other investigators (1–14). Patients with lung metastases have been reported to have both a good (3–7) and a poor (1,2) prognosis, with our results suggesting an intermediate prognosis. Patients with boney metastases have also shown an intermediate prognosis (4,9).

Recently, murine melanoma tumors have been shown to have heterogeneity, with subpopulations of cells with diverse biologic behavior exhibiting selective patterns of metastases (15–19). Our data suggest that the distribution of metastases in human malignant melanoma may not be random. Illmensee and Mintz (19) have shown that murine melanocytes in early development home to predictable organ sites; perhaps the initial distribution of melanoma metastases is related to this observation.

The patterns of metastatic melanoma can be compared to information known about breast cancer 20–30 years ago when clinical patterns of metastatic disease were initially recognized, but little prognostic information or predictive assays were available. It is now general knowledge that patients with metastatic breast cancer have a relatively good prognosis if the skin or lymph nodes are the initial prominent site of involvement. Patients with breast cancer with pulmonary nodules or boney metastases have an intermediate prognosis between those patients with skin or nodal involvement and those with liver or brain metastases and occur most often in women whose tumors are estrogen receptor- and progesterone receptor-positive. Women with breast carcinoma metastatic to the liver or brain or who have estrogen receptor- and progesterone receptor-negative tumors have a poor prognosis (25).

We suggest that there are three general groups of patients with metastatic melanoma that differ in clinical behavior and responsiveness to therapy. Good predictive biologic markers do not presently exist for melanoma; nevertheless, future clinical trials should consider these three metastatic patterns in their treatment design.

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Phase I Trial of Mitoxantrone in Children[1]

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Mitoxantrone was administered as a single iv injection once every 3 weeks to 84 children with advanced acute leukemia and solid tumors in a phase I trial. Dose-limiting granulocytopenia occurred at dosages > 18 mg/m² in children with solid tumors, while hospitalization for febrile episodes occurred in nine of 12 patients with acute leukemia receiving dosages > 24 mg/m². Six children developed evidence of cardiac dysfunction, including three instances of congestive heart failure. No other significant toxicity was noted. Complete responses were seen in one child with neuroblastoma metastatic to bone, one with acute lymphoblastic leukemia, and four with acute nonlymphoblastic leukemia. [Cancer Treat Rep 69:403–407, 1985]

Mitoxantrone, an anthraquinone derivative (1) developed in the search for cytotoxic intercalating agents devoid of cardiotoxicity, has antitumor activity in animal systems (2) without evidence of anthracycline cardiotoxicity in the rodent (3), dog, or monkey (4). Phase I testing in adult solid tumor patients indicated that leukopenia was dose-limiting, occurring at doses > 12 mg/m² administered every 3–4 weeks (5). As pediatric patients often tolerate higher doses of anticancer drugs than do adults (6), a phase I trial specifically directed toward the pediatric age group was undertaken.

METHODS

Patient Selection

Children ≤ 18 years of age with a histologically confirmed diagnosis of malignancy were eligible provided that their disease was refractory to conventional therapy. Informed consent was obtained in all cases. Criteria for entry in this study included the following: life expectancy of 4 weeks, recovery from the toxic effects of all prior chemotherapy or radiotherapy, adequate hematologic function in children without marrow malignancy (granulocyte count ≥ 1000/μl, platelet count ≥ 75,000/μl), adequate renal function (creatinine < 1.5 mg/dl or creatinine clearance > 80 ml/minute/1.73 m²), adequate hepatic function (bilirubin < 2.0 mg/dl and normal prothrombin time), and normal cardiac function.

Treatment Schedule

Mitoxantrone was supplied as a sterile blue solution of the free base at a concentration of 0.5 mg/ml in 5- and 10-ml ampules by the American Cyanamid Company and the National Cancer Institute. The drug was infused over 30 minutes in 100 ml of 5% dextrose in water once every 3 weeks.

To avoid putting patients in the study at biologically inactive dose levels, the anticipated starting dosage of 1.2 mg/m² (derived as one-third the toxic dose low in the beagle dog) was bypassed in favor of the actual starting dosage of 7.2 mg/m², which had been demonstrated to be a mildly myelosuppressive dose in a then-ongoing trial of the drug in adults (5).

The second dosage level was 10 mg/m², with subsequent escalations of 2 mg/m² to be consistent with the adult trial. A minimum of three patients (at least two

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with solid tumors and at least one with leukemia) were entered at each dosage level until hematologic toxicity identified the maximum tolerated dose for patients with solid tumor. Subsequent dosage levels accrued a minimum of three patients with leukemia. There were no dosage escalations in individual patients.

Study Parameters

Pretreatment evaluation included complete physical examination with tumor measurement when possible. Laboratory studies included hematologic profile, SMA-12 chemistry panels, coagulation studies, urinalysis, ECG, and echocardiogram. Symptoms and physical findings, as well as hematologic, hepatic, and renal functions, were assessed weekly during the study. Cardiac function was evaluated prior to every third course. Patients were considered evaluable for toxicity if they received one course of mitoxantrone with at least 3 weeks of follow-up, and were removed from study in the event of progressive disease or unacceptable toxicity. In July 1981, the study was amended to require cardiac nuclear scans if the patient had received > 350 mg/m^2 of prior anthracycline therapy.

Laboratory and radiographic examinations pertinent to tumor response were performed prior to each course. For patients with solid tumors, complete response was defined as total disappearance of tumor, while partial response was defined as a $\geq$ 50% decrease in the size of all measurable disease. For patients with leukemia, a complete marrow response was recorded if blasts decreased in the bone marrow to $\leq$ 5% in association with normal bone marrow cellularity, while partial response reflected reduction to within 5% and 25% blasts in the bone marrow.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	84
Male/female	50/34
Prior treatment	84
Chemotherapy	84
Chemotherapy and radiotherapy	41
Disease	
Acute lymphoblastic leukemia	28
Acute nonlymphoblastic leukemia	17
Myeloblastic	9
Monocytic	3
Myelomonocytic	4
Undifferentiated	1
Solid tumor	39
Neuroblastoma	11
Ewing's sarcoma	8
Osteosarcoma	7
Rhabdomyosarcoma	4
Wilms' tumor	2
Chondrosarcoma	2
Synovial sarcoma	1
Malignant fibrous histiocytoma	1
Embryonal carcinoma	1
Hepatoblastoma	1
Primitive neuroectodermal tumor	1

RESULTS

Eighty-four children $<$ 19 years of age were entered in the study; patient characteristics are shown in table 1. All but one of the patients had received prior anthracycline therapy. Table 2 shows the number of children with solid tumors and acute leukemia entered at the various dosage levels, as well as the number of early deaths (prior to Day 21) and the incidence of nonhematologic toxicity.

TABLE 2.—Entry on phase I study of mitoxantrone*

Dose level (mg/m^2)	Total entered	Solid tumor	Leukemia	Early death†	Nonhematologic toxicity			
					Infection‡	Nausea and vomiting	Mucositis	Cardio-toxicity
7.2–8.0	7	4	3	0	1	1	0	2
10.0	6	4	2	2	1	1	0	0
12.0	5	3	2	1	1	1	0	0
14.0	5	3	2	1	0	0	0	0
16.0	6	2	4	0	1	0	0	0
18.0	20	10	10	3	4	0	0	1
20.0	13	9	4	0	0	1	0	0
22.0	6	4	2	2	2	2	1	1
24.0	4	0	4	0	1	2	0	2
26.0	4	0	4	0	2	1	0	0
28.0	3	0	3	0	2	1	0	1
33.0	5	0	5	1	5	0	3	0
	84	39	45	10	20	10	4	7

* Unless otherwise specified, values = No. of patients.
†Within 21 days of being placed in the study.
‡Requiring hospitalization.

TABLE 3.—Mitoxantrone cardiotoxicity

Mitoxantrone dose level (mg/m^2)	Toxic effect*	Change in LVSF (%)†	Cumulative mitoxantrone (mg/m^2)	Cumulative doxorubicin (mg/m^2)	Diagnosis‡
8.0	DLVSF	36 → 25	28	490	ALL
18.0	CHF	30 → 15	126	525	Chondrosarcoma
22.0	CHF	29 → 13	116	117	Neuroblastoma
24.0	DLVSF	31 → 26	90	153	ANLL
24.0	DLVSF	36 → 21	142	153	ANLL
28.0	CHF	34 → 18	187	142§	ANLL

* DLVSF = decreased left ventricular shortening fraction.
†LVSF = left ventricular shortening fraction.
‡ALL = acute lymphoblastic leukemia; ANLL = acute nonlymphoblastic leukemia.
§Daunorubicin.

Some of these patients were previously reported (7).

Five of the early deaths occurred in children with solid tumors, four in children with acute lymphoblastic leukemia and one in a child with acute nonlymphoblastic leukemia. Median time to death was 12 days (range, 5-16). In five of these patients, early death was thought to be due to progressive disease. Three patients with leukemia had suffered CNS hemorrhage, while the cause of death was uncertain in two. No death was thought to be directly drug-related. The child with acute nonlymphoblastic leukemia had clearing of the blasts from the peripheral blood prior to death.

Hospitalizations for infections or fevers of unknown origin occurred at all dosage levels. Of the 20 children hospitalized, all but one had acute leukemia. Nine of the 12 children with acute leukemia receiving $\geq$ 26 mg/m^2 of mitoxantrone every 3 weeks required hospitalization for fever of unknown origin. Nausea and vomiting were noted in ten patients and occurred at all dosage levels. A 9-year-old child with acute lymphoblastic leukemia had mild nausea and vomiting prior to receiving mitoxantrone and died 17 days later with intractable nausea and vomiting. The remaining nine patients had nausea and vomiting only on the day that they received mitoxantrone. In two of these children, the symptoms were recorded as severe. Mucositis was noted in four patients, one at a dose of 22 mg/m^2 and three at 33 mg/m^2; this occurred 10-12 days after receiving mitoxantrone.

Six children developed evidence of cardiac dysfunction. Their characteristics and amount of prior anthracycline therapy are shown in table 3. Three of these children manifested changes in their echocardiogram, which reverted to normal following cessation of mitoxantrone. Three patients developed symptoms and signs of congestive heart failure (CHF), which were controlled by digitalis and diuretics; the clinical criteria for CHF included cardiomegaly, tachypnea, dyspnea, tachycardia, and hepatomegaly, supported by cyanosis, edema, or hypertension. Two of these patients developed CHF 1 and 3 months following cessation of mitoxantrone therapy. One of these patients has been previously reported (8). None of these patients had received other anticancer therapy after mitoxantrone, prior radiation to the heart, or was receiving iv therapy at the time CHF was diagnosed. One patient had received a transfusion of 250 ml of packed rbcs 1 week earlier. The median cumulative mitoxantrone dosage delivered to the 84 patients in the study was 28 mg/m^2 (range, 10-187); of the seven patients receiving the highest cumulative doses (90-187 mg/m^2), five exhibited evidence of cardiotoxicity.

Twenty-eight solid tumor patients evaluable for hematologic toxicity received 42 courses of mitoxantrone.

TABLE 4.—Myelosuppression by mitoxantrone in children with prior radiotherapy (XRT) or malignant marrow invasion

Dose (mg/m^2)	No. of patients/ No. of courses	Nadirs ($\times 10^3$ cells/μl)		Comment*
		Granulocytes	Platelets	
7.2	1/2	0.680, 0.620	5, 28	Marrow positive for neuroblastoma
14.0	1/2	0.252, 0.484	132, 223	XRT to pelvis and lung
16.0	1/2	0.168, 0.810	116, 175	Marrow positive for neuroblastoma
16.0	1/1	0.240	117	XRT to pelvis and spine
18.0	1/2	1.000, 0.700	36, 96	XRT to pelvis, hemiabdomen, and lung
18.0	1/1	0.660	73	XRT to abdomen and mediastinum
20.0	1/1	0.420	188	XRT to abdomen, thorax, and lung

* All patients also received prior chemotherapy.

TABLE 5.—Myelosuppression by mitoxantrone at each dose level

Dose (mg/m^2)	No. of patients/ No. of evaluable courses	Median nadirs ($\times 10^3$cells/μl)	
		Absolute granulocyte count (range)	Platelet count (range)
7.2-8.0	3/5	1.792 (1.140-4.836)	238 (91-500)
10.0	3/7	1.728 (0.798-6.360)	396 (180-620)
12.0	3/5	1.400 (1.200-3.162)	227 (50-304)
14.0	2/3	1.170 (0.599-1.739)	240 (132-570)
18.0	4/10	1.143 (0.414-1.960)	250 (112-330)
20.0	4/7	0.520 (0.296-2.100)	208 (91-500)
22.0	2/5	0.049 (0.005-0.195)	163 (121-404)

Seven patients with either marrow disease or extensive radiotherapy to marrow-bearing areas were analyzed separately (table 4). Three patients developed thrombocytopenia, while all seven became neutropenic. In addition, six of these seven patients had decreased levels of hemoglobin, ranging from 2.0 to 4.8 g/μl.

The myelosuppressive toxic effects sustained by the remaining 21 patients are presented in table 5. Granulocytopenia (absolute granulocyte count < 1000/μl) was the acute dose-limiting toxic effect of mitoxantrone. As noted in table 5, the degree of granulocytopenia was related to the dosage of mitoxantrone administered. The time to occurrence of the granulocyte nadir was 8-16 days, with recovery by Day 21-28 in all patients, except for one entered at the 22-mg/m^2 level who required up to 35 days for recovery. There was no consistent thrombocytopenia noted at the dosage levels of mitoxantrone administered to these children. Four children receiving dosages $\geq$ 18 mg/m^2 sustained decreases of 2.0-3.9 g/dl in their hemoglobin levels.

The antitumor effects of mitoxantrone were assessed in 74 children. There were two responses among 34 children with solid tumors: a partial response in a child with Ewing's sarcoma treated at 16 mg/m^2, and a complete response in a child with neuroblastoma metastatic to bone treated at 22 mg/m^2. Among 24 children with acute lymphoblastic leukemia, there was one complete response lasting 88 days in a child treated at 33 mg/m^2, and no partial responses. There were four complete responses, lasting 21-98 days, and three partial responses among 16 children with acute nonlymphoblastic leukemia. The complete responses were observed at 16, 26, 28, and 33 mg/m^2, while the partial responses were recorded at 24, 24, and 28 mg/m^2.

DISCUSSION

The results of this study indicate that mitoxantrone is a potentially useful anticancer agent in children. The acute dose-limiting toxic effect was myelosuppression, as has been noted in adults; children with solid tumors, however, in the absence of marrow disease or prior radiotherapy to marrow-bearing areas, tolerated higher doses of mitoxantrone than did their adult counterparts [18 mg/m^2 in children *versus* 12 mg/m^2 in adults (5)]. Children with leukemia, in whom hematologic parameters were not evaluable, tolerated still higher doses without significant toxicity to other organ systems, although hospitalization for febrile episodes became more frequent as dosages were escalated.

Cardiac dysfunction was the other major toxicity observed in this study, and appeared to be associated with cumulative doses of mitoxantrone. It should be noted that all but one of the patients received prior anthracycline therapy, making it difficult to estimate the relative contribution of previous *versus* current cardiotoxic therapy. Our study suggests, however, that increasing cumulative doses of mitoxantrone and/or increasing duration of mitoxantrone therapy subsequent to anthracycline therapy are closely associated with the development of cardiac dysfunction. Cardiac dysfunction has been observed in adults treated with mitoxantrone, with a 3% (24 of 801 patients) overall incidence in phase II trials of this agent conducted by the Southwest Oncology Group (9). Twelve of these adult patients had congestive heart failure.

Mitoxantrone is active against childhood acute nonlymphoblastic leukemia, as evidenced by seven responses among 16 patients. It has previously demonstrated activity in adult acute nonlymphoblastic leukemia (10). Although mitoxantrone is seemingly less active in childhood acute lymphoblastic leukemia and solid tumors, this patient population was heavily pretreated with anticancer agents, including other anthracyclines, and was exposed to levels of mitoxantrone which were often well below the maximum tolerated doses. As the drug has activity against adult acute lymphoblastic leukemia (11) and solid tumors (12), it therefore remains the task of phase II studies to evaluate the activity of mitoxantrone in children. The recommended starting dosages for such trials are 18 mg/m^2 every 3 weeks for patients with solid tumors, based on the dose-limiting toxicity of myelosuppression seen at 20 mg/m^2, and 24 mg/m^2 every 3 weeks for patients with leukemia, based on the increased frequency of hospitalization for fever of unknown origin seen at doses above this level.

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Activity of JM9 in Advanced Ovarian Cancer: A Phase I–II Trial[1]

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Thirty-nine patients with advanced solid tumors, including 28 with ovarian cancer, were entered in a phase I–II trial of a new platinum analog, JM9. Twenty-three patients had received prior chemotherapy which did not include cisplatin. Based on preliminary information from an ongoing study, our starting dose was 180 mg/m^2. The total dose of JM9 was administered in 1 L of saline infused over 1 hour, with no additional hydration or electrolyte supplementation. Courses were repeated at 3-week intervals or after full recovery from thrombocytopenia. One hundred thirty-nine courses (range, one to six per patient) were administered at four dose levels: 180 mg/m^2 (13 courses); 240 mg/m^2 (64 courses); 300 mg/m^2 (45 courses); and 350 mg/m^2 (17 courses). The dose-limiting toxic effect was thrombocytopenia, which was dose-related and cumulative. Median platelet count nadirs were 50, 47, 25, and 28 × 10^9/L for previously treated patients at dose levels of 180, 240, 300, and 350 mg/m^2, respectively. For patients who had not received prior chemotherapy, the corresponding values were 403, 61, 44, and 36 × 10^9/L. The nadir was predictable at Day 14 with recovery by Day 21 in earlier courses, but with delay of recovery to Days 28–42 in later courses and at higher dose levels. Twenty-five courses of chemotherapy in 15 patients were associated with a platelet count nadir of < 20 × 10^9/L, but despite this, serious hemorrhage was rare. Leukopenia was dose-related and mild; the median wbc count (× 10^9/L) was 2.2 (range, 1.0–7.3) at the highest dose level of 350 mg/m^2. The leukocyte count nadir was later than that for the platelet count (Days 21–28), and recovery was often not complete by the time of retreatment. All patients showed a progressive rise in mean corpuscular volume in successive courses, often accompanied by a fall in hemoglobin. Transfusions were required in 14 patients, 12 of whom had received prior chemotherapy. Nausea and vomiting, starting within 1 hour of drug administration, occurred in all patients, but appeared to be less severe and prolonged compared to that occurring with cisplatin. Diarrhea occurred in most patients at the two higher dose levels. There was no evidence of significant renal impairment, electrolyte disturbance, hearing loss, or peripheral neuropathy. Two patients had mild allergic reactions shortly after drug infusion and two others developed vasculitic rashes which were self-limiting. Responses were only seen in patients with ovarian cancer: six complete and two partial responses were achieved in ten patients who had not received prior chemotherapy, and one complete and three partial responses were achieved in 16 patients who had previously been treated with alkylating agents. Second-look laparotomies were performed in six complete responders, three of whom were pathologically disease-free. [Cancer Treat Rep 69:409–416, 1985]

An intensive search for new platinum-based compounds which retain the antitumor activity of cisplatin, but lack some of its more serious side effects, has led to the synthesis of a quadrivalent derivative, JM9 (CHIP) (fig 1).

Cisplatin has undergone extensive clinical evaluation

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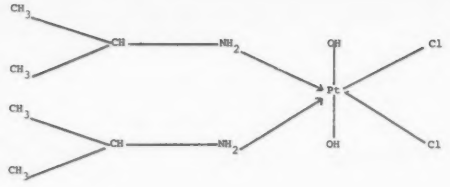

FIGURE 1.—Structure of JM9 [*cis*-dichloro-*trans*-dihydroxy-bis-isopropylamine platinum(IV)].

and is particularly active against testicular teratoma (1), but carcinomas of the ovary, bladder, cervix, and head and neck, as well as osteosarcomas, are also responsive (2-7). Unfortunately, the drug has many toxic effects. The most important of these effects is nephrotoxicity which is dose-related, cumulative, and often partially irreversible. Dose-related myelosuppression, cumulative ototoxicity (manifest as high-frequency hearing loss), and peripheral neuropathy also occur. Nausea and vomiting, poorly controlled by antiemetics, are other troublesome side effects.

In experimental animal models CHIP has generally shown comparable antitumor activity to cisplatin, giving an increase in lifespan > 100% in L1210 and P388 leukemias, B16 melanoma, Lewis lung carcinoma, mammary tumors of BDF mice, and colon 26 and 38 tumors. It is cross-resistant with cisplatin in L1210 but is active against L1210 resistant to carmustine and cyclophosphamide (8,9).

Preliminary data, using the in vitro human tumor clonogenic assay system of Hamburger and Salmon, suggest that there is little cross-resistance between cisplatin and CHIP (10).

The prevalent toxic effects of CHIP in mice, rats, and dogs, at ⩾ LD50, were myelosuppression and gastrointestinal effects. Changes in hepatic and renal functions were only noted at higher dose levels (11). When administered to mice at doses equivalent to 80% of the calculated LD50, CHIP produced negligible nephrotoxicity, in contrast with significant rises (up to 19 times the resting level) in blood urea seen after cisplatin (12).

CHIP shows enhanced water solubility in comparison with cisplatin (44.1 vs 8.9 mM) (13), and preclinical studies have demonstrated more rapid excretion with little nephrotoxicity. In the rat, after a dose of 10 mg/kg, the β-half-life was 14 hours for CHIP compared to 69 hours for cisplatin (14). For the dog, the β-half-life was 39 hours (15).

The plasma and renal pharmacokinetics of CHIP have been studied in man by Pendyala et al (16). Total platinum, ultrafilterable platinum species, and intact parent drug were measured. In 13 patients studied over a dose range of 20-270 mg/m^2, plasma decay of total platinum was biphasic, with an α-half-life of 0.4-2.2 hours and a β-half-life of 58-108 hours. Studies using radiolabeled platinum have compared the pharmacokinetics of cisplatin, CHIP, and carboplatin. The blood clearance of CHIP, like that of cisplatin, had two exponential phases; the mean fraction of radioactivity excreted in the urine in the first 6 hours was 52% for CHIP compared with 26.4% for cisplatin. These findings were interpreted as indicating less organ retention and less potential for nephrotoxicity for CHIP than for cisplatin (17).

MATERIALS AND METHODS

Criteria for Eligibility

Patients entered in the early stages of this study had advanced, histologically proven solid tumors, which were no longer suitable for conventional treatment. Patients with ovarian cancer, who had received no prior chemotherapy, were entered at higher dose levels after responses had been observed in this tumor type. Patients were between 26 and 69 years of age, with Karnofsky performance scores of > 40%, and had recovered from the major toxic effects of prior therapy. No patient had received cisplatin chemotherapy. Other criteria for entry in the study included adequate hematologic values (wbc count ⩾ 4.0 × 10^9/L; platelet count ⩾ 100 × 10^9/L) and hepatic excretory function (serum bilirubin ⩽ 20 μmols/L) and renal function (serum creatinine ⩽ 120 μmols/L; creatinine clearance ⩾ 70 ml/minute). Most patients had progressive disease, measurable by clinical examination and/or radiology, and all gave informed consent.

Trial Design

CHIP was administered as a single iv dose, repeated at 3-week intervals, or after full recovery from thrombocytopenia (platelet count ⩾ 100 × 10^9/L). Information available from the early stages of a phase I study using a similar schedule (18) suggested that 180 mg/m^2 was a safe starting dose. The dose was escalated by three increments to 350 mg/m^2. After reaching the maximum tolerated dose, subsequent patients were entered at the two intermediate dose levels (240 and 300 mg/m^2) in an extended phase I-II study to obtain further information about cumulative toxicity and response in ovarian cancer. Responding patients received a maximum of six courses of chemotherapy. Patients were not re-entered at higher dose levels.

Therapeutic Regimen

CHIP was administered as an iv infusion given over 1 hour, with no pre- or post-hydration. The drug was supplied by Bristol-Myers International Corp, Brussels, Belgium, as a lyophilized cake, and was reconstituted with

0.9% sodium chloride at a concentration of 1 mg/ml. The total dose was diluted in 1 L of 0.9% sodium chloride prior to infusion. All patients received antiemetic therapy with conventional doses of metoclopramide (10 mg every 4 hours), and most were sedated with lorazepam (2–4 mg).

Pretreatment and Follow-up Investigations

Baseline studies included history and physical examination; Karnofsky performance status; tumor measurements; cbc with differential wbc count; biochemical profile including electrolytes, serum creatinine, uric acid, calcium and magnesium, 24-hour creatinine clearance, and urinary protein; chest radiograph; ECG; and bone marrow. Most of the patients with ovarian cancer had abdominopelvic computed tomography (CT).

Cbcs were performed twice weekly and biochemical tests, including creatinine clearance, were performed at weekly intervals. Twenty-four-hour urinary protein excretion was analyzed on Days 5–7. In patients with ovarian cancer, CT was repeated after three and six courses.

Pure-tone audiometry and speech discrimination tests were performed prior to each course of CHIP and at the conclusion of therapy. Bithermal caloric tests (after Dix and Hallpike) of vestibular function were performed in most patients.

Urine samples were collected prior to drug administration and 24 and 48 hours after treatment, and urinary albumin, α_1-microglobulin (α_1-m), β_2-microglobulin, retinol binding protein, and α_1-acid glycoprotein were measured by single radial immunodiffusion using monospecific antisera and corresponding protein standards (19). Creatinine was measured by the method of Jaffe. To eliminate the effect of urine flow rate, the protein levels were expressed as milligrams of protein per gram of creatinine.

Definitions of Response and Toxicity

Criteria for clinical response and grades of toxicity used in this study are those defined by the World Health Organization (20). Response was also documented by abdominopelvic CT in patients with ovarian carcinoma and by second-look laparotomies in selected patients.

Statistical Methods

The influence of CHIP therapy on the levels of platelet counts, leukocyte counts, serum creatinine, 24-hour urinary creatinine clearance, serum magnesium, and calcium was examined. The analysis took the form of applying Friedman's nonparametric two-way analysis of variance to data across courses and within courses, one factor being time and the other being the individual patient. If this was statistically significant, multiple Wilcoxon matched-pairs sign-ranked tests were performed using a reduced significance level to locate the difference. Friedman's test was applied at $\alpha = 0.05$ significance level and the Wilcoxon test at $\alpha = 0.05$ divided by n, where n equals the number of patients to whom the test was applied.

RESULTS

Characteristics of the 39 patients entered in this study are shown in table 1, and numbers of patients included and courses of chemotherapy administered at each dose level are shown in table 2.

Thrombocytopenia, which was dose-related and cumulative, was the dose-limiting toxic effect (table 3). At a dose of 180 mg/m^2, two poor-risk patients developed marked thrombocytopenia (platelet counts of 33 and 38 $\times$ 10^9/L), but myelosuppression did not occur in the other two patients. At the three higher dose levels thrombocytopenia occurred in all patients, although occasionally it was not apparent until the second or third course. The nadir was predictable at Day 14, with recovery by Day 21 in earlier courses, but with delay of recovery to Days 28–42 in later courses and at higher dose levels. Ten patients received six courses of chemotherapy, six at 240 and four at 300 mg/m^2. Cumulative toxicity was manifest as lower platelet count nadirs and delayed recovery in successive courses and was more marked at 300 mg/m^2 (table 4). When platelet count nadirs in successive courses were compared for each of these ten patients, a statistically significant difference between Course 1 and the remainder was demonstrated. Changes in platelet count nadirs between Courses 2 and 6 did not reach

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	39
Sex	
Male	4
Female	35
Median age in yrs (range): 52 (26–69)	
Median Karnofsky performance score (range): 80% (40%–100%)	
Previous treatment	
Prior chemotherapy	23
Single alkylating agent*	11
Sequential single alkylators*	6
Other single agent†	3
Multiple agents	3
Prior radiotherapy	10
Limited	4
Extensive	6
Chemotherapy and radiotherapy	8
No previous chemotherapy or radiotherapy	11‡

* Cyclophosphamide, melphalan, or chlorambucil.
†Diaziquone × 4 courses (2 patients); high-dose etoposide (1 patient).
‡Ovarian cancer, 7 patients.

TABLE 2.—Tumor types

Type	No. of patients
Ovary	28
Serous cystadenocarcinoma	
Well-differentiated carcinoma	7
Moderately differentiated carcinoma	3
Poorly differentiated carcinoma	4
Mucinous cystadenocarcinoma	2
Endometrioid carcinoma	
Well-differentiated carcinoma	2
Poorly differentiated carcinoma	5
Mesonephroid carcinoma	1
Undifferentiated carcinoma	2
Fallopian tube adenocarcinoma	2
Miscellaneous	11
Renal carcinoma	2
Colon carcinoma	2
Unknown primary carcinoma	4
Thymus carcinoma	1
Breast carcinoma	1
Lung (squamous) carcinoma	1

statistical significance. Because of small numbers, separate analysis according to dose was not possible. No patient received more than three courses at 350 mg/m^2, and at least one platelet count nadir $< 20 \times 10^9$/L occurred in all patients receiving five to six courses at 300 mg/m^2. Twenty-five courses of chemotherapy in 15 patients were associated with a platelet count nadir $< 20 \times 10^9$/L. Despite this, serious hemorrhage was rare. Minor bleeding from nose and gums, petechiae, and occasional ecchymoses were noted in eight patients. After the fifth course one patient (No. 4) with an intra-abdominal mass from ovarian carcinoma, which may have infiltrated the bowel, developed gastrointestinal hemorrhage (when her platelet count fell to 15×10^9/L) associated with impaired renal function. After transfusion of platelets and 7 units of blood, her marrow and renal function slowly recovered, but she died suddenly 3 weeks later. A second patient with complete bowel obstruction became severely thrombocytopenic (platelet count, 7×10^9/L) 5 days after CHIP; her blood pressure and hemoglobin dropped and she died a few hours later. Permission for postmortem examination was withheld in both patients.

In five patients dose reductions were made because of thrombocytopenia. The platelet count nadirs at each dose level are shown in table 4. With the exception of Patient 10, who had received no prior radiotherapy or chemotherapy (but was treated at the highest dose level of 350 mg/m^2), all had received prior alkylating agent therapy, which in three patients was oral melphalan.

Leukopenia was generally mild (table 5) and there were no serious infections. Only one patient (No. 4) experienced a wbc count nadir of $< 1.0 \times 10^9$/L and a neutrophil count of 0.16×10^9/L. The leukocyte count nadir occurred later than that for platelet count (Days 21–28),

TABLE 3.—Hematologic toxicity: thrombocytopenia*

Dose (mg/m^2)	No. of patients	No. of evaluable courses†	No. of toxic courses‡	Platelet count					
				Count, $\times 10^9$ cells/L		Day of nadir		Day of recovery	
				Median	Range	Median	Range	Median	Range
180									
Prior chemotherapy	3	9	5	38 (38)	8–251	14	10–28	35	28–42
No chemotherapy	1	3	0	363 (363)	368–412	—	—	—	—
240									
Prior chemotherapy	10	28	21	47 (58)	9–287	14	7–17	14	21–42
No chemotherapy	7	35	28	58 (127)	14–269	14	10–28	21	21–56
300									
Prior chemotherapy	6	20	18	36 (66)	3–165	14	14–21	28	21–49
No chemotherapy	5	22	21	46 (66)	5–101	14	14–21	21	21–49
350									
Prior chemotherapy	4	10	8	31 (34)	5–130	14	14–21	21	21–35
No chemotherapy	3	7	10	53 (53)	14–82	14	14	21	21

* Values in parentheses = median platelet count nadir, Course 1 only.
†No nadir blood cell counts for 5 courses; 12 courses given at reduced dose included in relevant dose level.
‡Platelet count $\leq 100 \times 10^9$cells/L.

TABLE 4.—Hematologic toxicity: cumulative thrombocytopenia

	Course No.—						
	1	2	3	4	5	6	Recovery
	*240-mg/m² dose**						
Platelet count nadir (× 10^9cells/L)							
Median	98	60	57	43	46	46	
Range	42-269	23-140	15-115	17-99	25-90	14-128	
Average course delay (wks)	—	0.3	0.5	0.8	1.7	2.3	2.0
	300-mg/m² dose†						
Platelet count nadir (× 10^9cells/L)							
Median	86	42	44	25	18	14	
Range	72-150	31-75	30-58	16-57	13-35	5-26	
Average course delay (wks)	—	0.3	1.3	1.0	2.0	2.5	3.0

* Six patients. Only 1 patient had received previous chemotherapy.
†Four patients.

and recovery was often not complete at the time of retreatment. In some patients mild to moderate leukopenia (wbc count 1.5-3.0 × 10^9/L) persisted throughout the later courses, recovering slowly at the end of treatment. In general, the neutrophil count followed the total wbc count and rarely fell to < 50%. In six patients, absolute neutrophil counts between 0.5 and 1.0 × 10^9/L were recorded, usually in later courses, at the three higher dose levels. This did not seem to relate to prior chemotherapy (three were previously untreated).

All patients showed a progressive rise in mean corpuscular volume with successive courses. Serum B12, serum and rbc folate were measured if the mean corpuscular value rose to > 100 fl, but were normal in nine of ten patients and borderline low in one. Transfusions were given if the hemoglobin fell to < 8 g/dl and were required in 14 patients, 12 of whom had received prior chemotherapy.

Nausea and vomiting starting with 1 hour of drug administration occurred in all patients and usually continued for 4-8 hours, with eight to 12 episodes. Most patients had anorexia and some nausea for an additional 2-3 days but persistent vomiting was infrequent. Standard antiemetics (metoclopramide, 10 mg every 4 hours; prochlorperazine, 5 mg every 4 hours) ameliorated but did not abolish the vomiting. The severity and duration of vomiting appeared to be dose-related. However, with small numbers of patients at each dose level, individual variability could be a confounding influence. Diarrhea occurred briefly in 23 patients. Moderate to severe diarrhea occurred in 14 of 18 patients at 300 and 350 mg/m², but was mild to moderate and easily controlled by premedication with codeine phosphate (30-60 mg, as required) in nine of 17 patients at 240 mg/m².

With the exception of one poor-risk patient (No. 4) who developed reversible prerenal failure due to dehydration (maximum serum creatinine, 0.59 mmol/L at 8 days, falling to 0.13 mmol/L at 16 days), there was no evidence of significant renal impairment. Serum creatinine did not rise to > 0.13 mmol/L, and although creatinine clearance values were sporadically low, their timing did not relate to treatment, and these were probably due to inadequate urine collections. Proteinuria, up to 6 g/L between Days 5 and 7, has been reported for another platinum analog, TNO-6 (21,22); although minor increases in protein excretion of 0.1-0.4 g/24 hours were recorded in 14 of our patients in some courses, there was no evidence this was persistent or cumulative. There were no significant changes in serum magnesium or calcium.

TABLE 5.—Hematologic toxicity: leukopenia*

	Dose (mg/m²)			
	180	240	300	350
No. of patients	4	17	11	7
No. of courses	12	63	42	17
Wbc count nadir (× 10^9cells/L)				
Median	3.1	2.9	2.1	2.3
Range	0.7-5.8	1.5-6.7	1.3-6.3	1.0-7.3
No. of courses with—				
≥ 4.0 × 10^9 wbcs/L	4	13	7	2
≤ 2.0 × 10^9 wbcs/L	1	5	12	7

* One patient had wbc count nadir of 0.7 × 10^9cells/L.

Information on urinary α_1-m and albumin excretion at 0, 24, and 48 hours was available for 34 patients. Other urinary proteins β_2-microglobulin, retinol binding protein, and α_1-acid glycoprotein were assayed in some patients but did not provide additional information. α_1-m levels of < 5 mg/g of creatinine were considered normal, 5-15 mg/g was considered borderline, > 15 mg/g was considered abnormal, and for albumin < 30 mg/g was considered excessive excretion. In 23 patients receiving 61 courses of CHIP, α_1-m was normal or borderline. Albumin excretion was intermittently raised in a few patients, but could not clearly be related to treatment and may have been due to contamination of urine by vaginal secretions. Minor increases (30 mg/g of creatinine) in α_1-m at 48 hours were observed in three patients.

In six patients, ranging over all four doses, α_1-m was found to be variably elevated, but this could not clearly be related to CHIP chemotherapy. Hypercalcemia, preexisting tubular dysfunction, and rapid tumor progression with general debility may have been contributing factors. For two patients treated at the 350-mg/m^2 dose, raised levels of α_1-m at 24 and 48 hours were observed in Courses 1 and 2, with persisting increases prior to Course 3. This may have indicated minor tubular dysfunction.

Ten patients received six courses and two patients received five courses of CHIP, with no evidence of peripheral neuropathy. Serial audiometry was performed in 37 patients but posttreatment assessments were missing in nine, six of whom were too ill to attend due to disease progression. Although a few patients had pre-existing ear disease and some had age-related high-tone fall-off, no significant drug-related changes in hearing or vestibular function could be detected in the remaining 28 patients. One patient complained of vertigo but detailed neurological and vestibular function tests were normal and this symptom was felt to be psychogenic in origin and subsequently resolved spontaneously.

Two patients developed a brief itchy erythematous rash shortly after infusion of CHIP. This occurred during the third course in one patient who was not rechallenged since she had progressive disease. The other patient, who was responding to chemotherapy, received four additional courses without incident. Two patients developed vasculitic rashes, with a purpuric component, on the shins between Days 7 and 14. These rashes were self-limiting, occurring in Courses 2 and 4 of five in one patient and in Courses 1 and 2 of three in the second patient. At a time when her disease was regressing, one patient developed mild abnormalities of liver function (raised alanine and aspartate aminotransferases, glutamyl transpeptidase, and alkaline phosphatase) accompanied by CT scan changes of a fatty liver which have persisted but not deteriorated as treatment continues.

Thirty-seven patients were evaluable for tumor response to CHIP. There was one early death and one patient had no measurable disease. Overall, there were seven patients with complete remissions (CR), five with partial remissions (PR), two with minor responses (< 50%), ten with stable disease, and 13 with disease progression. Major responses were only seen in patients with ovarian cancer. Eight of ten of these patients had not received prior chemotherapy and four of 16 had been previously treated; their responses to CHIP are summarized in table 6. CRs were based on clinical and CT criteria. Responses were usually rapid, but CT scans to document CR were only performed at 3 and 6 months. Thus, durations of response (CR and PR) have been recorded from the start of chemotherapy. Second-look laparotomies have been performed in six complete responders. Three laparotomies were negative and these patients remain in remission 12, 16, and 26 months from the start of chemotherapy. Three patients had microscopic residual disease. One patient relapsed at 11 months in the chest and later in the abdomen, the second remains in remission at 24 months,

TABLE 6.—Response to CHIP

Patient No.	Type of response	Dose (mg/m^2)	Sites of disease	Method of measurement	Duration (mos)
			Prior chemotherapy (16 evaluable patients)		
1	CR*	240	Abdomen, pelvis	CT	11
2	PR	300	Abdomen, pelvis, ascites, effusion	CT, x-ray	8
3	PR	300	Pelvis	CT	9
4	PR	350 (→ 240)	Abdomen	Clinical, CT	5
			No prior chemotherapy (10 evaluable patients)		
5	CR†	240	Pelvis	Clinical, CT	26+
6	CR*	240	Abdomen, pelvis	Clinical, CT	8
7	CR†	240	Liver, abdomen, pelvis	Clinical, CT	12+
8	CR†	240	Abdomen, pelvis	Clinical, CT	16+
9	CR*	300	Pelvis	Clinical, CT	24+
10	CR	350	Abdomen, pelvis	Clinical, CT	9
11	PR	240	Abdomen, pelvis (bone)	Clinical, CT, x-rays	14
12	PR	300	Abdomen, pelvis (scar)	Clinical, CT	5

*Two microscopic seedlings and 1 macroscopic seedling at second-look laparotomy.
†Negative second-look laparotomy.

and the third relapsed in the abdomen at 8 months. The last patient had multiple peritoneal seedlings, whereas the first two had a single focus of disease. Most regressions have been in pelvic, omental, and nodal disease, although one patient showed healing of bone metastases. Documented liver metastases progressed in two patients. Responses were observed at three dose levels of 240, 300, and 350 mg/m^2. There was no clear relationship to histology, although two patients with adenocarcinoma of the fallopian tube both responded to CHIP.

DISCUSSION

Preclinical data suggested that the spectrum of toxicity of CHIP might differ from that of the parent compound cisplatin, but that antitumor activity would not be lost. An earlier phase I trial (18) using the same schedule established that the dose-limiting toxic effect in man was thrombocytopenia and that CHIP had little potential for nephrotoxicity. Two other phase I studies using different schedules also found thrombocytopenia to be the dose-limiting toxic effect (23,24). This current study confirms and extends these data. Myelosuppression was dose-related and predictable at doses > 240 mg/m^2, thrombocytopenia being more severe than leukopenia. Since a number of patients responded to chemotherapy, it was possible to accrue new data about the cumulative effects of CHIP. Platelet count nadirs were generally lower with successive courses and their recovery was slower. Thus, although doses of 300 mg/m^2 at 3-week intervals were initially well-tolerated in previously untreated patients, severe prolonged thrombocytopenia often occurred in later (fourth to sixth) courses.

Where possible, responding patients were continued at the same dose levels, but platelet count nadirs in later courses were usually in the range of 10–30 × 10^9/L. Since platelet counts < 50 × 10^9/L may be associated with hemorrhage and the effects of CHIP are cumulative, dose reductions for nadirs below this level are probably advisable. Further falls in platelet count nadirs were usually prevented by dose reductions of 15%–25%.

We recommend a dose of 300 mg/m^2 every 4 weeks for phase II studies in patients who have not received prior chemotherapy. A new course should not be initiated until full platelet count recovery (> 130 × 10^9/L), and a 20% dose reduction is recommended if the platelet count nadir is < 50 × 10^9/L in the previous course. A starting dose of 240 mg/m^2 is advised for heavily pretreated or poor-risk patients. CRs have been observed at both of these dose levels in patients with ovarian cancer.

CHIP is clearly less nephrotoxic than cisplatin. After an initial response, one elderly lady with ovarian cancer deteriorated, becoming confused and dehydrated during her fifth course of CHIP. A rise in serum creatinine to 0.59 mmol/L was corrected by iv fluids, although she eventually died of progressive tumor. Although patients were carefully instructed and were generally cooperative in collecting 24-hour urine for creatinine clearance, social pressures and occasional amnesia reduced the reliability of serial values for this test. Patient error or mechanical factors (eg, vesicovaginal fistula) accounted for many sporadic low values, and there was no pattern of deterioration related to chemotherapy. There were no significant abnormalities of urinary protein excretion in 23 patients. Minor rises in α_1-m excretion in three patients and more significant abnormalities in two other patients indicative of tubular dysfunction may have been related to CHIP therapy. In the remaining patients, other factors such as hypercalcemia and debility were contributory factors. These minor changes, detected by sophisticated tests, are in sharp contrast with the severe proteinuria seen after TNO-6 (22) and the well-documented renal damage (25,26) seen when cisplatin is given without hydration. Cisplatin tends to produce mild tubular proteinuria when given with adequate hydration (27).

Peripheral neuropathy, usually dose-related, is a less common but disabling complication of cisplatin treatment (28), and it is encouraging that this problem was not encountered in 39 patients, 12 of whom received high total doses of CHIP. Similarly, high-frequency hearing loss, which is dose/schedule-related and cumulative (29), is frequent after cisplatin, but careful serial testing revealed no deterioration in auditory function in 38 patients.

Electrolyte disturbances, in particular hypokalemia, hypocalcemia, and hypomagnesemia, are common after cisplatin therapy (30,31) but were not encountered in this study.

Although troublesome, the gastrointestinal effects of CHIP were less severe than those of the parent compound. After high-dose cisplatin, nausea and vomiting commonly persist for up to 1 week, whereas emesis rarely lasted > 24 hours following CHIP and patients were eating normally within 2–3 days. Although not mentioned in the phase I study from Roswell Park Memorial Institute (Buffalo, NY), diarrhea occurred in 60% of our patients. Although severe and explosive at 350 mg/m^2, it was of brief duration and easily controlled by codeine phosphate at doses ≤ 300 mg/m^2.

Most patients entering this study had ovarian cancer; CHIP is highly active against this disease, particularly in patients who have received no prior chemotherapy. There are a few studies of cisplatin as a single agent in previously untreated patients, but Blackledge and Luesley (32) have reported an overall response rate of 68% (clinical CR, 56%) using 100 mg/m^2 every 3 weeks for five doses. Given the small numbers in our study, this is similar to an overall response rate of 80% (clinical CR, 60%) described here. A 25% response rate in pretreated patients receiving CHIP is also in the same range as that for cisplatin. This suggests that the spectrum of activity may be similar to that of cisplatin, but further phase II stud-

ies, in a broad range of tumor types, are required. Patients who had received cisplatin were not entered in this study, so we are unable to comment on possible cross-resistance between CHIP and the parent compound.

In conclusion, CHIP is a promising new platinum analog which is highly active against ovarian cancer. Advantages over the parent compound, cisplatin, include lack of nephrotoxicity, electrolyte disturbance, peripheral neuropathy, and ototoxicity. At the highest dose level nausea and vomiting seemed considerably less severe and of shorter duration than after cisplatin, but this needs careful comparison in a randomized study. The dose-limiting toxic effect is cumulative thrombocytopenia, which may present problems when CHIP is used in combination with other myelosuppressive agents. Our recommended dose for phase II trials in previously untreated patients is 300 mg/m^2 every 4 weeks, modified to 240 mg/m^2 in heavily pretreated or poor-risk patients.

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Phase II Trials of Maytansine, Low-Dose Chlorozotocin, and High-Dose Chlorozotocin as Single Agents Against Advanced Measurable Adenocarcinoma of the Pancreas[1,2]

Gastrointestinal Tumor Study Group[2,*]

One hundred and five patients with advanced measurable pancreatic carcinoma were randomized to receive therapy with maytansine, low-dose chlorozotocin (120 mg/m^2), or high-dose chlorozotocin (175 mg/m^2). Objective response rates were as follows: maytansine, no responses among 48 patients; low-dose chlorozotocin, none among 27; and high-dose chlorozotocin, three among 30 (10%). Among patients with excellent performance status (Eastern Cooperative Oncology Group grade of 0–1) and no prior chemotherapy, response rates were as follows: maytansine, no responses among 17 patients; low-dose chlorozotocin, none among 14; and high-dose chlorozotocin, three among 28 (11%). The responses observed with high-dose chlorozotocin were transient (5–8 weeks) and were of no benefit to the patients. None of these agents given by the methods of this study can be recommended for patients with advanced pancreatic cancer. [Cancer Treat Rep 69:417–420, 1985]

The Gastrointestinal Tumor Study Group (GITSG) has undertaken a series of phase II studies of patients with measurable metastatic pancreatic cancer. The initial trials consisted of a randomized comparison of single agents: dactinomycin, methotrexate, and doxorubicin (1). A subsequent study compared the current agents hexamethylmelamine, galactitol, razoxane, and BTGdR (2). Additionally, FAM (5-FU, doxorubicin, and mitomycin), SMF (streptozocin, mitomycin, and 5-FU), SAMe (streptozocin, doxorubicin, and semustine), and cisplatin were evaluated. In the performance of these trials, the GITSG made periodic adjustments in the method of patient selection and assignment to treatment. To provide an adequate evaluation of patients thought most likely to respond to treatment, these adjustments were designed to allow early closing of programs employing inactive drugs, and to ensure that at least 12 patients with both a performance status (PS) of 0 or 1 and no prior chemotherapy would be treated with each regimen.

The present study included the following single agents.

Maytansine, an ansa macrolide which inhibits mitotic spindle formation, has been demonstrated to have antineoplastic activity in cell culture and murine tumor systems. In limited phase I studies, responses have been noted in a variety of human tumors (3,4). Phase II studies have been conducted in advanced sarcomas (5), breast cancer (6,7), and cervical carcinomas (8).

Chlorozotocin, a water-soluble chloroethyl nitrosourea with the cytotoxic group attached to the C-2 position of glucose, was utilized to circumvent the delayed hematologic toxicity that limited the clinical use of other nitrosourea agents. Chlorozotocin has been demonstrated to have antineoplastic activity in murine systems and has undergone extensive phase I testing (9–11).

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MATERIALS AND METHODS

Patients were considered eligible if they had histologically confirmed advanced measurable adenocarcinoma of the pancreas of ductal, acinar, or undifferentiated variety. Patients with islet cell and cystadenocarcinoma were excluded. The source of the primary lesion had to be proven by either surgical inspection or a clearly defined radiographic defect of the pancreas. To assess the possibility of response or progression, the tumor had to be measurable by palpation or x-ray. A liver scan could be used if it demonstrated a clearly defined perfusion defect of at least 5 cm in greatest diameter. Sonography or computerized tomographic scanning were not accepted as methods for assessing measurable disease. Hepatomegaly was considered a suitable indicator lesion if the liver contained proven metastases and was palpable at least 5 cm below the xiphoid or midclavicular costal margin on quiet respiration. Patients were required to have not had any abdominal surgery involving resection or bypass for at least 3 weeks; any abdominal exploration including biopsy for 2 weeks; any previous radiation therapy or chemotherapy for 1 month; and any treatment with a nitrosourea for at least 7 weeks.

Patients who had failed prior chemotherapy or radiation therapy were eligible. Patients were required to have adequate bone marrow function, including a wbc count $> 4000/mm^3$ and a platelet count $> 100,000/mm^3$. Patients were ineligible for participation in the study if they had: active infection, acute pancreatitis, prior malignant tumors (other than basal or squamous cell carcinoma), severe anorexia, nausea or frequent vomiting, or caloric intake < 1200 calories per day. All patients were fully informed about risks, limitations, and side effects of the treatment, and were provided with a written consent document as required by institutional and Federal guidelines.

Study parameters before treatment included: a complete medical history, physical examination, height, weight, body surface area, routine urinalysis, hemoglobin, wbc count, platelet count, chemistry profile, prothrombin time, fasting blood glucose, chest x-ray, and carcinoembryonic antigen assay. Serial follow-up procedures included: a weekly cbc and a complete hematological and chemical profile at the time of each new course of therapy. Chest x-ray, other radiologic studies, and CEA assays were performed at 2-3-month intervals. Treatment was continued for a minimum period of 6 weeks. Patients with disease progression at or after 6 weeks could be assigned to an additional treatment arm. Patients receiving a second protocol treatment are classified as crossover patients and are only included in this report for the evaluation of response. Patients continued in the study until there was evidence of disease progression or symptomatic deterioration, after which follow-up continued to ascertain survival time.

Criteria for response were as follows. *Complete response* was defined as the disappearance of all measurable lesions observed at least 6 weeks after the beginning of therapy. *Partial response* was defined as a decrease of $\geq 50\%$ in the product of the largest perpendicular diameters of the most clearly measurable area of malignant disease, with no increase in the size of other known areas of malignant disease and no new areas of malignant disease. This result was required to persist at least 6 weeks after the beginning of therapy. If hepatomegaly represented the indicator lesion, there had to be a decrease of at least 30% in the sum of the measurements beneath the xiphoid process and both costal margins at the midclavicular line. *Disease progression* was defined as an increase of $\geq 25\%$ in the original measurements of the product of the two largest perpendicular diameters of the tumor, or the appearance of new areas of malignant disease. All responses were subject to joint review by group members. Survival was calculated from the date of initiation of drug treatment.

Individual Treatments

Maytansine

Maytansine was administered by rapid iv push at a dosage of 0.6 mg/m^2/day. The same dosage schedule was repeated at 3-week intervals for three courses: on Days 1, 2, and 3 (first course); Days 22, 23, and 24 (second course); and Days 43, 44, and 45 (third course). Following this, if the patient was objectively stable or had shown an objective response, maintenance therapy would be administered at 4-week intervals. Leukocyte and platelet counts were obtained weekly. A reduction of 33% of the dose was required if the wbc count was $< 2000/mm^3$ or the platelet count nadir was $< 75,000/mm^3$. Moderate or severe gastrointestinal side effects (nausea, vomiting, or diarrhea) resulted in a reduction in the dose of 20% and 40%, respectively. If moderate or severe neurotoxicity (paresthesia, muscle weakness) occurred, provision was made for a dose reduction of 20% or 40%, respectively.

Provision was also made for a 20% dose reduction if the SGOT rose by 200%-300%, and chemotherapy was delayed if there was an increase > 300%. An increase of direct serum bilirubin to a value of 1.3-2.0 mg/dl occasioned a 50% decrease in drug dosage, and a value > 2.0 mg necessitated delay in administration of the drug. If toxicity was not encountered, escalation of the dosage by 20% was possible.

Chlorozotocin

The low-dose schedule for chlorozotocin was 120 mg/m^2 by rapid iv injection every 6 weeks, while the high-dose regimen was 175 mg/m^2 by rapid iv injection every 6

TABLE 1.—Patient entries and response results

Drug	No. of patients	Response		
		No. of responders	Duration (wks)	Survival (wks)
Maytansine				
PS 0–1	17	0		
PS 2	10	0		
PS 3	6	0		
Crossover	15	0		
All patients	48	0		
Chlorozotocin (120 mg/m^2)				
PS 0–1	14	0		
PS 2	6	0		
PS 3	3	0		
Crossover	4	0		
All patients	27	0		
Chlorozotocin (175 mg/m^2)				
PS 0–1	28	3	5,8,7	16,26,118
PS 2	0	0		
PS 3	2	0		
Crossover	0	0		
All patients	30	3		

weeks. Because of its instability in aqueous solution, the drug was administered immediately after reconstitution. For patients who had delayed thrombocytopenia < 75,000/mm^3 or leukopenia < 2500/mm^3 after the initial dose, subsequent doses were reduced to 80 and 125 mg/m^2 for the low- and high-dose regimens, respectively. In patients receiving the low-dose regimen, provision was made for dose escalation to 150 mg/m^2 if there was no leukopenia or thrombocytopenia after two courses of therapy.

RESULTS

Table 1 shows analyzed patient entries and response results. Eight patients have been excluded from analysis because of nonmeasurable lesions, and one was excluded because the tumor was an islet cell tumor. An additional seven patients were not treated after entry. Thus, there was a total of six, six, and five patients who were excluded from analysis with maytansine, low-dose chlorozotocin, and high-dose chlorozotocin, respectively. Eleven

TABLE 2.—Toxic effects associated with treatment

Toxic effect	% of patients		
	Maytansine (n = 33)	Chlorozotocin 120 mg/m^2 (n = 33)	Chlorozotocin 175 mg/m^2 (n = 30)
Hematologic (cells/mm^3)			
Wbc count nadir			
2000–2999	9	4	10
1000–1999	3	0	3
< 1000	0	0	0
Platelet count nadir			
50,000–129,999	15	4	30
25,000–49,999	0	0	3
< 25,000	0	0	10
Severe vomiting	6	4	0

patients in the maytansine group, eight in the low-dose chlorozotocin group, and no patients in the high-dose chlorozotocin group had received prior chemotherapy.

Objective responses were observed only in the high-dose chlorozotocin arm (three of 30 patients), and response duration was uniformly short. The duration of response and survival times for the three responding patients, as measured in weeks, are shown in table 1. Median survival times for the treatment groups were 9.3, 10.6, and 8.3 weeks for maytansine, low-dose chlorozotocin, and high-dose chlorozotocin, respectively.

Table 2 details some of the observed toxic effects. Hematologic toxicity, especially leukopenia and thrombocytopenia, occurred with high-dose chlorozotocin and maytansine. Vomiting, which subsequently occurred, was more common with maytansine. Other side effects included paresthesia of fingers and toes in one patient and severe anorexia in two patients receiving maytansine, and severe anorexia in one patient receiving low-dose chlorozotocin. Fatal septicemia and endocarditis occurred in one patient receiving high-dose chlorozotocin.

DISCUSSION

Among the single agents employed in this study, maytansine and low-dose chlorozotocin showed no evidence of therapeutic activity, and high-dose chlorozotocin produced only a minimal response rate with short durations of response. These unfavorable results were obtained despite efforts to include treatment of patients with excellent performance status and no prior chemotherapy. These agents do not warrant further study in pancreatic cancer by the methods we employed.

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Application of Biochemical Modulation With a Therapeutically Inactive Modulating Agent in Clinical Trials of Cancer Chemotherapy[1,2]

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Biochemical modulation refers to the administration of an agent to manipulate the metabolic locus of action of an antitumor agent so as to result in the selective enhancement of the latter's antitumor effect. The former agent is termed the "modulating agent," and the drug whose antitumor effects are enhanced is considered the "effector agent."

At the laboratory level, many examples of useful biochemical modulation have been reported. In contrast to these exciting experimental findings, clinical trials employing biochemical modulation have not clearly demonstrated improvement in the therapeutic indices of the effector agents. This review finds that these divergent results appear to be due to differences in the way the laboratory studies and clinical trials have been conducted. Further, this report suggests that changes in the design of clinical trials involving biochemical modulation may permit therapeutic success in the clinic.

The clearest example of successful biochemical modulation in the laboratory, followed by failure to reproduce similar results in the clinic, is that of the combination of PALA and 5-FU (FUra). FUra is believed to produce its antitumor effects by either or both of the two following mechanisms: (*a*) the inactivation of thymidylate synthetase by FdUMP by outcompeting its natural pyrimidine metabolite, dUMP; and/or (*b*) the formation of fraudulent (FUra) RNA by the incorporation of FUTP in place of the natural pyrimidine metabolite, UTP (1). In either mechanism, the intracellular depletion of tumor pyrimidine pools can enhance the anticancer activity of FUra. Thus, PALA, an inhibitor of de novo pyrimidine synthesis (2-9), favors the competition of FUra for its metabolic targets by lowering the levels of the normal pyrimidine nucleotides that compete with FUra. The above biochemical mechanisms were documented and demonstrated responsible for enhancement of the anticancer activity of FUra in both in vitro and in vivo laboratory tumor models (10-15). In the laboratory, varying dosage ratios of PALA and FUra (ie, moderate-dose PALA:high-dose FUra or high-dose PALA:low-dose FUra) were all found to be effective in enhancing antitumor activity in comparison to concomitant controls receiving the maximum tolerated dose (MTD) of FUra as a single agent.

In the clinical studies with PALA and FUra, concomitant single-agent FUra controls were not done. Comparison was with single-agent FUra historical controls. In these historical studies, the MTD of FUra as a single agent was 15-20 mg/kg (600-800 mg/m^2) iv once weekly (16,17) or 12 mg/kg (480 mg/m^2) iv daily for five doses followed by 6 mg/kg on alternate days until toxicity, followed by weekly doses of 15 mg/kg (18). However, in published clinical studies of PALA/FUra combinations (19-22), relatively high doses of PALA were used, and, under this condition of PALA administration, patients only tolerated much less than the optimal historical doses of FUra. For example, in a PALA/FUra trial which initiated two FUra dose schedules, the initial doses of FUra (480 mg/m^2 weekly and 400 mg/m^2/day for five doses) were reduced by one-half in 80% of patients because of intolerable toxicity (19). It was concluded in another study that the MTD of the PALA/FUra combination was approximately two-thirds of the MTD of each agent when used alone (21), and a more recent report (20) recommended only 200 mg/kg/day of FUra in combination for only 4 days every 3 weeks. Thus, in contrast to the laboratory studies where relatively moderate-dose PALA: high-dose FUra ratios were found equally active as high-dose PALA:low-dose FUra combinations, only high-dose PALA:low-dose FUra ratios were evaluated clinically and compared to FUra historical controls with FUra at its MTD. This high-dose PALA:low-dose FUra combination did not evidence better therapeutic results in the clinic than FUra alone at its optimal historical MTD.

In comparing the positive laboratory results with the

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negative clinical results, one outstanding difference between these studies emerges. In the laboratory, the moderate doses of PALA that were employed in the combination were still capable of exerting effective anticancer activity when used alone against experimental tumors, whereas in contrast, in the clinic PALA alone is considered essentially therapeutically inactive (23–31). This negative clinical finding does not necessarily mean that PALA has no clinical antitumor activity, for entirely different endpoints are utilized for determining therapeutic activity in the drug screening animal models and in the clinic (34).

In the clinic, drugs are defined as clinically active if the drugs elicit tumor regression ($\geq$ 50% in at least 20% of evaluable patients). In marked contrast, inhibition of tumor growth is the criterion of effectiveness employed to define the same antitumor agents as active in laboratory tumor models. With different endpoints being used to identify anticancer activity in humans and in animals, it is not surprising for the anticancer activity of PALA to be perceived as positive in one system and negative in the other. PALA is inhibitory against advanced animal tumors, but even chemotherapeutically induced complete inhibition of tumor growth in patients is negative by clinical criteria. As stated earlier, this negative clinical determination does not necessarily mean that PALA is devoid of either biological (ie, antitumor) or biochemical activity. Suggestions of antitumor activity (as well as toxicity, ie, biochemical activity) were reported with PALA in clinical high dosage. Hence, since the empiric rules of clinical combination chemotherapy call for each of the agents in a combination to show antitumor activity as a single agent, it is not surprising that PALA was pushed to high dosage in the clinical combinations with FUra. This decision resulted in the second agent (FUra) being clinically tolerated only if administered in low dosage.

The empirically derived rules of clinical combination chemotherapy are based primarily on the perception of the heterogeneity of neoplastic cell populations as qualitative in nature. If a subset of cancer cells can be killed by drug A and a different subset by drug B, then it is expected that a tolerated A plus B drug combination should kill both A- and B-sensitive cells and result in at least an additive therapeutic effect. This "qualitative" perception of chemotherapeutic heterogeneity has clearly facilitated therapeutic progress. However, in addition to the qualitative, there is quantitative heterogeneity. A subset of cancer cells can possess a gradient of responsiveness to an agent. Under appropriate conditions (in vitro), a very great number of such cells can be shown sensitive to a high dose of this effector agent. However, under in vivo conditions, the low chemotherapeutic indices of most agents (FUra) can make the eradication of even slightly resistant cells impossible at in vivo tolerated drug doses. Thus, the use of a modulating agent (PALA) to selectively manipulate intratumoral biochemical mechanisms to amplify the tumor cytotoxicity of an effector agent (FUra) can facilitate the chemotherapeutic attack, even though the modulating agent itself appears to have no antitumor activity (15).

The published clinical trials with the PALA/FUra combination were conducted to determine whether biochemical modulation with PALA could improve FUra chemotherapy in the clinic. Since historical FUra controls were used, reason indicates that the dose of the effector agent, FUra, in the PALA/FUra combination should be the same as (or as close as possible to) the FUra dose used in the historical studies; ie, the MTD, or at least a high dose, of FUra. Reason also indicates that the dose of the modulating agent (PALA) need only be one capable of appropriate biochemical activity (and not necessarily biological activity, such as antitumor activity). Thus, the ratio of the two agents should likely be that of low-dose PALA:high-dose FUra. Use of a dose of the modulating agent high enough to require a significant reduction of the dose of the effector agent (when the latter agent is at its MTD in the historical controls) is likely to be self-defeating in comparison with the historical studies employing high-dose therapeutic activity of the effector agent.

There is other reasoning that can be employed to reach the conclusion that a low-dose PALA:high-dose FUra ratio should be employed in the PALA/FUra clinical trials. Skipper and Schabel (32) have noted that "None [of the animal models for solid tumors] . . . will save the experimental and clinical investigator the pain of having to learn to 'translate' in both directions. Translation means the changes in regimen required to achieve a similar response in a second tumor system when a given response to some therapeutic procedure is observed in one tumor system . . . ." Note that the call is to "translate," not transpose, the laboratory findings to clinical trial. It would appear that the laboratory reports of a beneficial therapeutic interaction between PALA and FUra merely led to attempts to transpose the combination to the clinical arena on the basis of the empirically derived rules of clinical combination chemotherapy described earlier, hence, the high-dose PALA:low-dose FUra ratio. However, translation "in both directions," particularly as called for above, leads to the following reasoning. Since PALA has no discernible therapeutic activity in the clinic, we restudied the problem in the laboratory to determine whether a low, nontherapeutic dose of PALA, but one still capable of exerting biochemical modulation, could enhance the therapeutic activity of FUra without undue toxicity. We found that a low, nontherapeutic dose of PALA, chosen on the basis of demonstrated biochemical effects at that dose, could selectively potentiate the antitumor effects of FUra (15). On the basis of these laboratory findings, a phase I clinical trial determined that a low dose of PALA (250 mg/m^2) could effectively inhibit pyrimidine synthesis in man. This low dose of PALA was

evaluated in combination with varying doses of FUra. It was found that this low PALA dose could be administered 24 hours before FUra at its MTD of 600 mg/m^2 once weekly without causing undue toxicity (33). This, then, is the low-dose PALA:high-dose FUra ratio that should be evaluated in patients to assess the antitumor activity of the PALA/FUra combination in comparison with historical results with FUra alone at its MTD.

A modulating agent that has only minimal clinical activity as a single agent is employed solely for its biochemical effects. The lowest dose of the modulating agent which effectively influences the metabolic locus of action of the effector agent in the tumor should be utilized in the clinic. Under these conditions, a therapeutically useful modulating agent will produce no, or minimal, changes in host tissues; therefore, the effector agent can be administered at or close to its MTD as a single agent. This is the clinical approach that properly tests the practical clinical utility of biochemical modulation of an effector agent with a therapeutically inactive modulating agent.

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Modulation of the Activity of PALA by Dipyridamole[1,2,3]

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PALA is thought to inhibit an early step in de novo pyrimidine synthesis, causing depletion of intracellular pyrimidine nucleotides. Dipyridamole, a nucleoside transport inhibitor which can block restoration of nucleotide levels via the salvage pathway, was tested for its ability to augment the cytotoxicity of PALA against normal and malignant human cells in vitro. At the clinically relevant concentration of 1 μM, dipyridamole increased the cytotoxicity of PALA against a melanoma, a colon carcinoma, a promyelocytic leukemia (HL-60), and normal marrow (CFU-GM) in clonogenic assays. Dipyridamole produced 50% inhibition of uridine uptake in these cells at concentrations of < 0.1 μM and reduced the LD50 of PALA by approximately 50% in mice. These results indicate that dipyridamole can markedly potentiate the activity of PALA in vitro and in vivo. [Cancer Treat Rep 69:425–430, 1985]

PALA is a stable transition-state inhibitor of aspartate transcarbamylase, the enzyme that catalyzes the second step in the de novo pyrimidine synthesis pathway (1,2). Inhibition of de novo pyrimidine synthesis results in depletion of pyrimidine ribonucleotide and deoxyribonucleotide pools (3), and this is associated with death of the treated cells. Many cells, however, can synthesize pyrimidine nucleotides using the "salvage pathway" when the corresponding nucleosides are available in the environment of the cell. Uridine has been shown to prevent the toxic and antineoplastic effects of PALA both in vitro and in vivo (2,4). Human plasma contains micromolar concentrations of pyrimidine nucleosides (5,6), and can potentially serve as a reservoir of nucleosides capable of antagonizing the cytotoxicity of PALA. Despite the fact that PALA reduces pyrimidine nucleotide levels in sensitive cells and tissues, the circulating level of uridine in patient plasma decreases only slightly after PALA treatment (5,6); the observed decrease may be due to accelerated uptake of uridine through increased activity of the salvage pathway (7,8).

Dipyridamole is a potent nucleoside transport inhibitor that binds to specific sites on the cell membrane (9) and blocks the facilitated uptake of many nucleosides and nucleobases (9–11). If the salvage of extracellular pyrimidine nucleosides is an important determinant of PALA activity, then concurrent treatment with a combination of PALA and dipyridamole should result in increased cytotoxicity. This study reports some of the findings on the effects of combining these two agents in the treatment of normal and malignant human cells in culture and in toxicity studies of PALA in mice.

MATERIALS AND METHODS

Cells and Cell Culture

Human promyelocytic leukemia HL-60 cells (12) were obtained from Dr. S. J. Collins (Veterans Administration Medical Center, Seattle, WA). Colon carcinoma (T-219), melanoma (T-242), and astrocytoma (T-24) cell lines were established in culture from human tumor xenografts growing in nude mice and were obtained from Dr. H. Masui (Nude Mouse Research Center, University of California-San Diego [UCSD], La Jolla).

All cell lines were routinely maintained under condi-

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tions of logarithmic growth in RPMI-1640 medium supplemented with 10% fetal calf serum (Irvine Scientific, Santa Ana, CA), 1% L-glutamine, and 1% antibiotic/antimycotic mixture (Grand Island Biological Co, NY).

Heparinized bone marrow aspirates were obtained from normal volunteers who were briefed on the risks of the procedure and had given their informed consent according to the UCSD Human Subjects Committee guidelines. The mononuclear cells from these samples were separated by Ficoll-Hypaque gradient centrifugation (13) and washed thrice in medium prior to experimental use.

Drugs and Chemicals

Dipyridamole was supplied by Boehringer Ingelheim Ltd (Ridgefield, CT); PALA was supplied by the National Cancer Institute (Bethesda, MD); and (5,6-^{3}H)-uridine (23.4 Ci/mmol) was obtained from New England Nuclear Corp (Boston, MA).

Inhibition of Uridine Uptake by Dipyridamole

Freshly harvested HL-60, colon carcinoma, melanoma, astrocytoma, and human bone marrow mononuclear cells were resuspended in RPMI-1640 medium (1×10^6 cells/2 ml for each experimental point). The samples were first incubated with or without dipyridamole at 0.01–10 μM for 30 minutes at 37°C under a 5% CO_2 atmosphere. ^{3}H-uridine at a final concentration of 10 μM was added and the incubation continued for an additional 30 minutes with occasional shaking. The samples were centrifuged at 4°C for 5 minutes at 500 × *g* and the supernate was discarded. The cell pellets were washed twice with 2 ml of cold phosphate-buffered saline, then resuspended in 900 μl of 0.1 N NaOH and maintained on ice for 10 minutes. A 500-μl aliquot of each resulting sample was then resuspended in 5 ml of Betaphase (West Chem Products, San Diego, CA) and the radioactivity was quantitated by liquid scintillation counting.

Clonogenic Assays

Human tumor cell lines were cultured in soft agar using the techniques of Hamburger and Salmon (14) and normal human granulocyte/macrophage progenitors were cultured as previously described (15). Cells were plated in 0.6% soft agar with 20% dextran/charcoal-treated (to remove nucleosides) fetal calf serum at a cell density of $1–5 \times 10^4$ cells/plate. The medium was supplemented with uridine to a final concentration of 10 μM. PALA at concentrations of 0, 5, 25, or 100 μM was added to the plates at the beginning of the culture, either with or without 1 μM dipyridamole. Plates were incubated at 37°C under a 7% CO_2 atmosphere and were scored for colonies of > 40 cells after approximately 2 weeks. All experiments were performed at least three times in triplicate. The stability of dipyridamole in the culture system was confirmed by direct measurement of dipyridamole concentration using high-pressure liquid chromatography (16).

Toxicity Testing

Athymic mice were obtained from the UCSD athymic mouse facility. They were divided randomly into the various PALA dose groups, and within each dose group, they were randomly assigned to receive either PALA alone or PALA plus dipyridamole. PALA was administered ip, and dipyridamole was given sc at a dose of 100 mg/kg. The numbers of animals surviving in each dose group at the end of a 30-day period were recorded.

RESULTS

A human colon carcinoma, a human melanoma, the HL-60 promyelocytic leukemia cell lines, and normal human bone marrow macrophage/granulocyte progenitors (CFU-GM) were cultured in soft agar with various concentrations of PALA alone or in combination with 1 μM dipyridamole. Both PALA and dipyridamole were present in the cultures during the whole 10–14-day period of colony formation. Figure 1A shows the dose-response curves for the inhibition of colony formation in the HL-60 promyelocytic leukemia and the T-242 melanoma cells by PALA. Each point represents the mean of three experiments performed in triplicate. Dipyridamole at a concentration of 1 μM reduced the number of colonies formed by T-242 to 87% ± 11% (SD) of control, and PALA alone was not very effective, reducing colony formation to only 73% of control at a concentration of 100 μM. In contrast, when dipyridamole was present, even 5 μM PALA produced > 80% inhibition of colony formation, representing a > 20-fold enhancement of PALA activity. In the HL-60 cells, dipyridamole by itself reduced colony formation to 80% ± 15% (SD) of control, and as expected from the fact that PALA is minimally myelosuppressive at maximally tolerated doses in humans, PALA treatment alone produced little reduction in the ability of HL-60 to form colonies. In the presence of 1 μM dipyridamole, there was approximately a twofold reduction in the number of colonies formed by the HL-60 cells at each PALA concentration.

Figure 1B shows the dose-response curves for the inhibition of colony formation by PALA in the normal marrow mononuclear cells (CFU-GM) and in the colon carcinoma cells (T-219). The curves are quantitatively similar to those shown in figure 1A, although the magnitudes varied with each cell type. The CFU-GM cells were more resistant than the HL-60 cells to the effects of PALA alone, with maximum inhibition at 100 μM not significantly different from control. In the presence of dipyridamole, however, almost complete inhibition of colony formation was observed at 25 μM. Less dramatic synergism was observed in the T-219 cells, where PALA alone inhib-

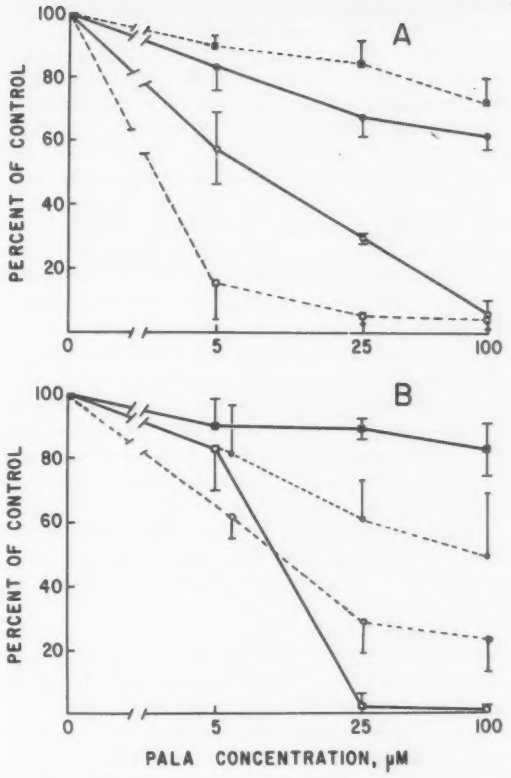

FIGURE 1.—Effect of PALA with and without dipyridamole on colony survival Panel A: Dose-response curves of the T-242 human melanoma to PALA alone (■) and in the presence of 1 μM dipyridamole (□). Also in the same panel are the HL-60 promyelocytic leukemia dose-response curves to PALA alone (●) and to PALA + 1 μM DP (○). Panel B: Dose-response curves of normal marrow mononuclear cells CFU-GM to PALA alone (■) and in the presence of 1 μM dipyridamole (□). Dose-response curves of the T-219 human colon carcinoma cells to PALA alone (●) and in combination with 1 μM dipyridamole (○) are also shown in this panel. Each data point represents the mean of 3 experiments each performed in triplicate, and the vertical bars represent 1 SD.

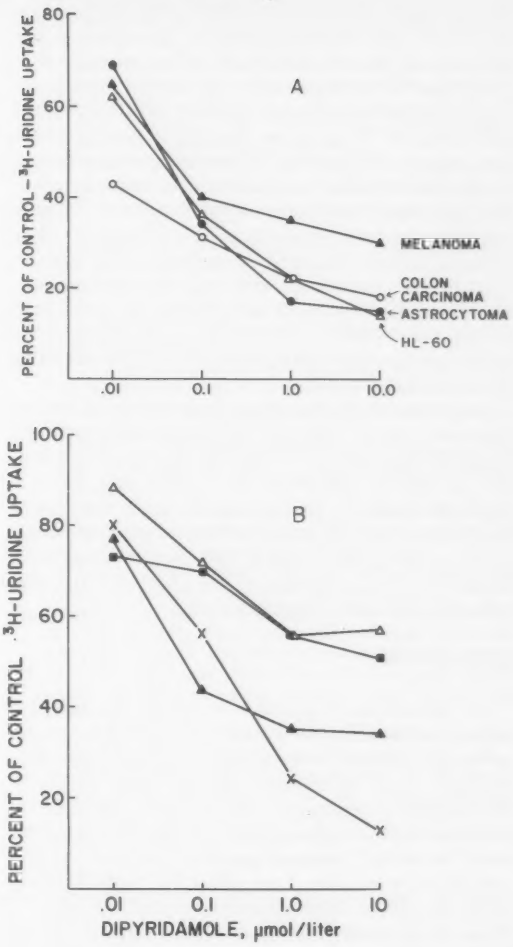

FIGURE 2.—Inhibition of ^{3}H-uridine uptake into malignant human cell lines (panel A) and into normal human bone marrow mononuclear cells (panel B) as a function of dipyridamole concentration. Each data point represents the mean of ⩾ 3 experiments performed in triplicate in panel A, and each curve represents a separate marrow sample in panel B.

ited colony formation to 55% of control, and dipyridamole augmented the inhibition approximately twofold at all PALA concentrations (two dashed lines). These results indicate that while dipyridamole is minimally cytotoxic by itself at clinically achievable concentrations (17,18), it markedly potentiates the activity of PALA against both normal and malignant human cells in culture.

To relate this synergism to inhibition of nucleoside transport, the effect of dipyridamole on the total cellular uptake of ^{3}H-uridine over a 30-minute period was measured. The uridine concentration in the cell environment during the experiment was 10 μM. Data are shown in figure 2A for each of the three malignant cell lines studied in the soft agar cloning assay as well as a human astrocytoma cell line. The uptake of ^{3}H-uridine was linear for all cell types from 1 to 30 minutes. It is important to emphasize that total cellular uptake is a parameter which reflects not only the transport of uridine but also its phosphorylation to nucleotides and its incorporation into macromolecules. Dipyridamole produced a 50% decrease in uridine uptake at concentrations < 0.1 μM for all four cell lines, but even at a concentration of 10 μM, inhibition was apparently not complete. Figure 2B shows that dipyridamole produced a similar pattern of inhibition of

uridine uptake into the mononuclear cells from four normal human bone marrow aspirates. The subject-to-subject variance, however, did not allow determination of an IC50. These data demonstrate that the 1 μM concentration of dipyridamole that is effective in enhancing the cytotoxicity of PALA is also capable of causing significant reduction in the ability of these same cells to assimilate uridine from their environment. Due to the nature of the experimental conditions, we could not observe a good correlation between the ability of dipyridamole to reduce uridine uptake and its ability to augment the in vitro activity of PALA in the different cell lines studied here. Since dipyridamole inhibits the uptake of many different nucleosides, the lack of correlation may also be due to the fact that nucleosides other than uridine are playing a role in antagonizing PALA.

In a group of five mice, the peak plasma concentration (geometric mean) was 11.5 μM (range, 4–17) and the plasma half-life was 5.1 ± 0.6 (SD) hours following a standard sc dose of 100 mg/kg of dipyridamole. Table 1 shows the impact of dipyridamole on the lethal dose of PALA for mice. In the absence of dipyridamole, the LD50 of PALA was in the range of 1000 mg/kg. When both drugs were given concurrently, the LD50 of PALA was reduced to between 400 and 500 mg/kg.

TABLE 1.—Toxicity of PALA without and with dipyridamole in nude mice

PALA (mg/kg ip)	Dipyridamole (100 mg/kg sc)*	
	Without	With
200	0/5	0/5
400	1/11	0/11
500	0/3	3/3
600	0/11	10/11
800	3/11	5/5
1000	3/6	Not done

* Values = No. of mice dying of toxicity by Day 30/No. treated at that dose.

DISCUSSION

The present study attempts to address the question whether inhibition of membrane transport of exogenous pyrimidine nucleosides could blockade the salvage pathway sufficiently to augment the cytotoxicity of PALA. Dipyridamole was selected as the membrane transport inhibitor not only because of its potency (9–11), but also because its use in thousands of patients as a vasodilator and antiplatelet agent for many years has established its safety in humans. Dipyridamole increased the ability of PALA to inhibit colony formation in all three of the malignant cell lines tested as well as in normal human CFU-GM cells. In vivo toxicity testing revealed that dipyridamole had a similar effect on the activity of PALA in mice, as evidenced by its ability in reducing the LD50 of PALA to approximately one-half of that observed in non-dipyridamole-treated animals. Although dipyridamole can cause other membrane changes (11,19), the most likely mechanism by which dipyridamole produced this modulation is through inhibition of membrane nucleoside transport (20). The implication is that both in culture and in the living animal, the salvage of pyrimidine nucleosides serves to antagonize the cytotoxicity of PALA.

Although no attempt was made at quantitation in any component of this study, nucleosides were believed to be available for salvage in all of the experiments reported here. Uridine concentrations of 1.9–11.8 μM have been reported in the serum or plasma in various strains of mice, rats, and man (5,6,21); cytidine is also present in similar concentrations (6). The medium used in our in vitro studies initially contained 10 μM uridine. Most cells seeded into soft agar die during the first several days of the culture (cloning efficiency < 1.0%), however, releasing a very complex mixture of nucleosides into the medium which are available for salvage by the few cells that survived to form colonies. Serial high-pressure liquid chromatographic analysis of culture supernates demonstrated the appearance of large peaks consistent with nucleosides in patterns so complex that individual compounds could not be quantified.

The argument that dipyridamole modulates PALA activity by limiting the transport of nucleosides is supported further by the recent results of Zhen et al (20). They demonstrated that a mixture of guanosine, cytidine, and deoxycytidine could prevent the toxicity of acivicin, another inhibitor of de novo pyrimidine synthesis. By inhibiting the uptake of these nucleosides, dipyridamole restored the activity of acivicin in culture against a murine hepatoma cell line. One point of difference between our results and those of Zhen et al is that in the absence of added nucleosides, they found that dipyridamole did not augment the activity of acivicin, whereas in our cultures dipyridamole did enhance the cytotoxicity of PALA. The difference is explainable by the fact that they cultured cells in nucleoside-free medium on plastic under conditions where there was a high cloning efficiency and very little cell death to cause the release of salvage pathway metabolites, whereas the massive cell death intrinsic to the soft agar assay provided a source of salvageable nucleosides in our cultures. Another possible explanation for the lack of augmentation to acivicin activity is that the hepatoma line used in those experiments was very sensitive to acivicin to begin with, and the 2 μM concentration used resulted in a > 90% reduction in colony counts, leaving very little room for further augmentation of acivicin activity by dipyridamole.

In the present study, the uptake of uridine was used as a measure of the ability of dipyridamole to interfere with nucleoside transport. The dipyridamole concentration producing 50% inhibition of uptake ranged from < 0.01 to 0.06 μM for the four malignant cell lines studied. It is

notable, however, that even at dipyridamole concentrations of 10 μM, uridine uptake was apparently not completely inhibited. This is consistent with the permeation of some uridine by diffusion rather than by the dipyridamole-inhibitable facilitated transport process (9), but the alternative explanation that some radioactivity remained associated with the cell as exchanged ^3H cannot be discounted. Several investigators have reported that uridine alone can protect cells from the cytotoxicity of PALA (2,4), and the mechanism of synergism reported here may work via uridine metabolism. The salvage of other nucleosides, however, is also thought to be important in determining the activity of PALA (3,20). More work in nucleoside salvage is needed before we could determine the mechanism of action of PALA/dipyridamole synergism.

Although several groups have attempted to use PALA as a biochemical modulator of other drugs such as 5-FU (22-24), the approach of using dipyridamole to augment the activity of PALA is novel and untested. PALA is active against slow-growing murine tumors such as B16 melanoma, Glioma 26, and Lewis lung carcinoma and inactive against the murine leukemias (25,26). In phase I (8,27-31) and II clinical trials against non-small cell lung cancer (32,33), colon cancer (34-36), melanoma (37), and head and neck cancer (38), PALA was found ineffective, with an overall response rate of only 3%. The difference in the activity of PALA against human versus murine tumors may be related to different levels of nucleosides in the plasma or differences in nucleotide metabolism between these two species. The clinical utility of the dipyridamole/PALA combination will depend on the ability of dipyridamole to differentially potentiate PALA activity in normal versus malignant tissues, but this combination provides a focused and novel approach that should be explored further before PALA is abandoned as an ineffective drug in man.

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Cold Protection and Heat Enhancement of Doxorubicin Skin Toxicity in the Mouse[1,2]

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A series of experiments were performed in a BALB/c mouse model to evaluate the efficacy of topical heating and cooling on doxorubicin (DOX) ulceration of the skin. Unanesthetized mice were administered a dose of 0.05 mg or 0.5 mg of DOX intradermally, followed by topical heating (43°C–44°C) or cooling (8°C–10°C) of the skin area for up to 1 hour. DOX disposition from skin and plasma was studied by high-pressure liquid chromatography in both cooled and uncooled groups of animals. Human tumor clonogenic cells were exposed to DOX for 1 hour at different temperatures to determine the direct effect of heat and cold on DOX-induced lethality in vitro. Skin temperature of 17°C ± 2.3°C was achieved with cooling and skin temperature of 38.5°C ± 1.2°C was achieved with heating, compared to control intradermal skin temperature of 32°C ± 0.5°C. Local heating caused duration-dependent DOX lethality: 20% after 20 minutes, 40% after 45 minutes, and 80% after 1 hour. There were no deaths in the control groups. A 20% lethality rate was constant in the cooled groups. Skin lesions were approximately fourfold larger in the heated groups receiving 0.5 mg of DOX ($P < 0.05$). In contrast, the application of cold significantly reduced intradermal DOX skin toxicity following the lower DOX dose of 0.05 mg ($P < 0.05$). There was no consistent benefit for cooling beyond a 45-minute duration, which achieved maximal protection against ulceration. Pharmacokinetic studies of DOX disposition in skin and blood failed to show a significant difference for total tissue concentrations or plasma levels between cooled and uncooled animals. However, clonogenic human tumor cells (HEC-1A endometrial cells) did demonstrate significantly reduced DOX effects when exposed to the drug at reduced temperatures. These results confirm the biologic efficacy of local cooling and clearly contraindicate the use of local heating to treat inadvertent DOX extravasations in the clinic. Instead, cold should be applied immediately and maintained for 45 minutes or longer to reduce or prevent serious DOX skin ulcerations in patients. [Cancer Treat Rep 69:431–437, 1985]

Inadvertent doxorubicin (DOX) infiltration into soft tissues can lead to severe ulceration and necrosis in patients. A variety of pharmacologic agents have been evaluated as local antidotes in laboratory animals. Some agents have shown protective effects, including low doses of corticosteroids (1), beta adrenergic compounds (2), adenosine (3), and dimethylsulfoxide with vitamin E (4). None of the compounds tested have been completely preventive and some have actually increased toxicity. Thus, the extravasation of DOX remains a serious clinical problem for which new therapies are needed. Barlock et al (5) recommend local corticosteroids and the application of an ice pack, while Larson (6) recommends only ice and limb elevation. Local heating, however, is probably the common method for the topical therapy of most medication-related phlebitis and some institutions (7) suggest this as a possible local remedy for DOX infiltration. Because of the confusion concerning temperature-related treatment approaches, the following series of experiments were performed to evaluate the separate application of heat and cold as a means of reducing DOX skin toxicity in an experimental mouse model.

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MATERIALS AND METHODS

Mouse Skin Toxicity

The mouse model for intradermal DOX has been described previously in detail (8). Adult female BALB/c mice (Jackson Labs, Bar Harbor, ME), weighing an average of 25 g, undergo the removal of dorsal hair from an area of 3 × 3 cm 24 hours prior to DOX injection. One to two applications of the topical depilatory agent Neet lotion (containing thioglycolic acid, mineral oil, calcium hydroxide, sorbitol, and steryl and cetyl alcohols) are used for this purpose. The experimental model uses intradermal injections of 0.5 and 0.05 mg of DOX (Adriamycin, Adria Labs, Wilmington, DE), each diluted into 0.05 ml of 0.8% sodium chloride, USP (without preservatives). Each injection is made into the dorsal skin of the mouse 24 hours after the hair-removal procedure.

A novel method was developed to restrain animals unanesthetized during heating and cooling. Cylindrical Plexiglas mouse restraints were modified by the removal of material on the top of the restraint to leave an access hole 2.5 cm in diameter. Above this access hole, a standard rubber prophylactic was suspended from a beaker rack so that the condom end fit snugly through the access hole. Immediately after the dorsal intradermal DOX injection, the mice were placed in the restraint so that the injected skin site was directly under the access hole. The open end of the restraint was then closed and the condom filled with water preheated to 45°C or an ice-water suspension of 3°C–5°C. The temperature of the solution was monitored by an electronic thermocouple probe immersed in the solution at the base of the condom. Another probe was inserted intradermally, proximal to, but not into, the intradermal DOX lesion. This allowed for serial measurements of intradermal skin temperature changes. After initial equilibration, solution temperature was maintained for various time intervals at 8°C–10°C for cooling experiments and 43°C–44°C for heating experiments. This was accomplished by repeated exchange with aliquots of newly heated or cooled water. All temperature measurements were made using a BAT-8 model digital electronic three-channel thermocouple thermometer using 29-gauge thermocouple needle probes (Bailey Instruments, Saddlebrook, NJ).

Initial experiments were performed to assess the serial intradermal skin temperature changes in control mice given intradermal DOX without subsequent heat or cold therapy. Sodium pentobarbital anesthesia was used in the first experiments instead of the modified cage restraints. This was abandoned due to a 3°C–5°C drop in skin temperature following the pentobarbital injection.

Heating or cooling was applied immediately after intradermal DOX and was maintained for selected time intervals up to 80 minutes. Since heating increased early DOX lethality, the heating duration was subsequently limited to 60 minutes. An additional cold treatment series evaluated a delay in the application of cold for 24 hours after intradermal DOX. Control groups included intradermal DOX alone at 0.05- and 0.5-mg doses (n = five mice each) and two groups receiving no DOX but heated and cooled as above for 60 minutes (n = three each).

Animals were divided into treatment groups of five each for similar treatments and caged together for the remainder of the experiment. The perpendicular widths of skin lesions were measured daily by micrometer. Three parameters of intradermal DOX toxicity were assessed: induration, erythema, and ulceration. In a previous report, these toxicity parameters were shown to increase in a doxorubicin dose-dependent fashion with respect to: (*a*) the total area of the lesion (the area under a toxicity-time curve or $cm^2 \cdot day$); and (*b*) the peak toxicity level (cm^2) and the duration of each toxic effect (days) (8). Only ulceration data were statistically analyzed since they were clearly the most important biologic variables in this antidote study.

Statistical analyses on individual animals were performed by computer for: (*a*) area under an ulcer size (centimeter squared) versus time (days) curve (AUC); (*b*) the peak lesion size (centimeter squared); (*c*) the total time of ulceration (days); and (*d*) the occurrence of treatment-related deaths and the duration of survival (days). These raw data, grouped according to treatment and DOX dose, were analyzed by the SPSS program ONEWAY. This provided an initial analysis of variance between similar treatment groups. A subsequent multiple range test, the Student Newman-Keuls (SNK) procedure, was then applied to segregate significantly different treatments into homogeneous subsets at a 0.05 level. Statistical significance was inferred when a particular treatment group was uniquely found in a subset different from that containing the control treatment. Since the number of statistically different subsets is not limited, rank-ordering of significantly different treatments is also possible with this procedure.

High-Performance Liquid Chromatographic (HPLC) Studies

Skin samples were excised from mice killed at serial times after receiving intradermal DOX and heating and cooling. Blood samples were removed by cardiac puncture from anesthetized mice prior to killing by rapid cervical dislocation. The skin tissue was homogenized by mortar and pestle under liquid nitrogen and then subjected to an organic extraction with chloroform/methanol (4:1). The same extraction method was used for the plasma samples. Extracted samples were dried under air and reconstituted with ammonium formate:acetonitrile (71:29), which served as the mobile phase for a reverse-phase HPLC assay based on the method of Israel et al (9).

Clonogenic Tumor Cell Studies

Human endometrial carcinoma cells (HEC-1A cell line supplied by Dr. J. Fogh, Memorial Sloan-Kettering Cancer Center, New York, NY) were exposed to DOX in vitro for 1 hour at different temperatures. The cells were then washed twice in media and plated at 4×10^4 cells/dish in soft agar using the two-layer clonogenic assay system of Hamburger and Salmon (10). Only colonies of ≥ 60 μm were counted 10–14 days after plating using an optical image analysis system optimized for tumor colony enumeration (FAS-II, Omnicon, Bausch and Lomb Inc, Rochester, NY).

RESULTS

Fourteen separate treatments, using only topical heat (43°C–44°C) or cold (8°C–10°C), were applied to groups of mice given 0.05 or 0.5 mg of intradermal DOX. Table 1 shows the mean data for these experiments. There were no treatment deaths in the control groups although characteristic intradermal DOX skin ulcerations did occur. There was also no skin ulceration in control animals receiving only topical heating or cooling for 60 minutes. Figures 1 (0.05 mg of DOX) and 2 (0.5 mg of DOX) show the characteristic skin toxicity patterns for the two intradermal DOX doses. Ulcerative toxicity in control animals lasted a mean of 20 days after 0.05 mg of intradermal DOX (fig 1) and 33 days after 0.5 mg of intradermal DOX (fig 2). In each instance, local heating increased ulceration, while cooling significantly reduced DOX ulceration. Local heating also caused duration-dependent lethality in the 0.5-mg intradermal DOX groups: 20% mortality after 20 minutes of heating, 40% after 45 minutes, and 80% after 1 hour ($P < 0.05$). Figure 3 shows the actual intradermal skin temperatures achieved proximal to the DOX lesions in heated and cooled mice. The average skin temperature in control mice receiving intradermal DOX was approximately 32°C ± 1°C. Mice exposed to the heated solution achieved a mean intradermal skin temperature of 38.5°C ± 1.2°C after an equilibration period of approximately 15–20 minutes. In contrast, when exposed to the cold solution, the intradermal skin temperature rapidly dropped to 15°C ± 4.3°C. This equilibrated slowly to an average of 17°C ± 2.3°C at the end of the 45-minute treatment period. Average core temperatures measured rectally at the end of each treatment series were 35.8°C for control mice, 36.1°C for heated mice, and 27.6°C for cooled mice.

The total ulcerative toxicity (AUC) in the heated groups was significantly increased after 45 minutes of heating following 0.5 mg of DOX and after 60 minutes of heating following 0.05 mg of DOX ($P < 0.05$). Peak lesion areas also increased fourfold from control values ($P < 0.05$).

In mice receiving post-DOX cooling, treatment-related lethality averaged one of five animals in each group. After 0.05 mg of intradermal DOX, cold produced statistically significant reductions in both ulcerative AUCs and peak lesion sizes. When cold was combined with the higher intradermal DOX dose of 0.5 mg, there were reductions in ulcerative AUCs, but these were not statistically significant. Delaying the application of cold for 24 hours was still beneficial for mice receiving the lower DOX dose ($P < 0.05$) but not with the higher dose. There was no lethality with the delayed cooling treatments fol-

TABLE 1.—Comparison of ulcerative skin toxicity following intradermal DOX (0.05 and 0.5 mg) and treatment with topical heat or cold (mean of 5 mice)

Topical therapy	Total toxicity AUC ($cm^2 \cdot day$)		Peak lesion area (cm^2)		Damage duration (days)		Therapy-related deaths (median days of death)	
	0.05 mg	0.5 mg	0.05 mg	0.5 mg	0.05 mg	0.5 mg	0.05 mg	0.5 mg
None (control)	0.674	9.264	0.210	0.540	13.0	33.0	0	0
Heat (43°C–44°C) Duration (mins)								
20	1.048	11.399	0.203	2.180*	10.4	34.6	1/5† (1.0)	1/5† (6.8)
45	ND‡	33.635	ND	2.20*	ND‡	37.0	ND‡	2/5 (7.0)
60	3.112*	0.567§	0.361	0.360§	11.33	8.8§	0	4/5 (1.0)*
Cold (8°C–10°C) Duration (mins)								
10	0.152*	6.768	0.024*	0.827	2.6	24.0	1/5 (7.0)	1/5 (3.0)
45	0.012*	6.201	0.008*	0.520	0.04	27.0	1/5 (1.0)	0
60	0.126*	5.333	0.017*	0.405	9.33	20.0	1/5 (1.0)	1/5 (1.0)
80	ND‡	9.316	ND‡	0.792	ND‡	28.4	ND‡	1/5 (2.0)
45 mins (delayed 24 hrs after DOX)	0.214*	12.853*	0.05*	1.078	4.5	30.2	0	0

* Denotes $P < 0.05$ difference from control for subset containing all similar treatments (hot or cold).

†No. of deaths/No. of mice treated.

‡Not done.

§Values lower due to early treatment-related deaths in most animals.

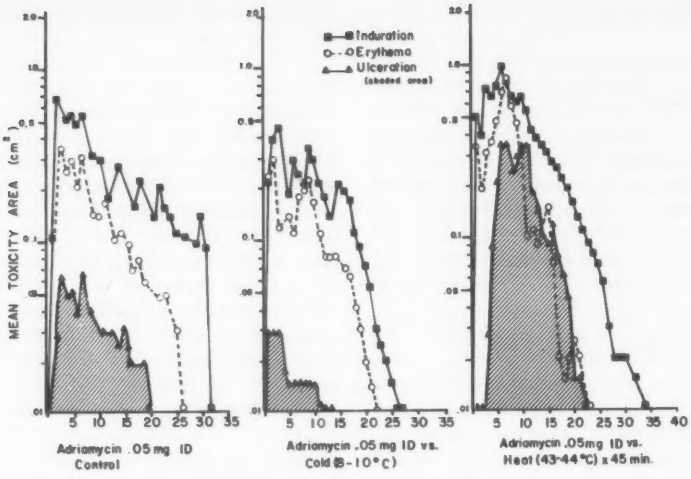

FIGURE 1.—Semilogarithmic plots of mean skin lesion areas (cm^2) vs time (days) for animals (n = 5 each) receiving 0.05 mg of intradermal (ID) DOX (control, left graph); 0.05 mg of intradermal DOX + 45 mins of topical cold, 8°C–10°C, applied immediately (middle graph); and 0.05 mg of intradermal DOX + 45 mins of topical heat, 43°C–44°C, applied immediately (right graph).

lowing either DOX dose.

There was no consistent relationship between the duration of cooling and the size of ulcers. In terms of the duration of visible ulceration following 0.05 mg of intradermal DOX, the following rank order of statistically different treatments was obtained (least toxic to most toxic): cold for 60 minutes < cold for 10 minutes < cold for 80 minutes < cold for 45 minutes delayed 24 hours < control < heat for 45 minutes ($P < 0.05$ by SNK multiple-range test).

The HPLC studies of DOX disposition in skin and plasma (fig 4, A and B) did not demonstrate significant differences in the concentrations of DOX in cooled animals. There was a trend toward reduced DOX skin concentra-

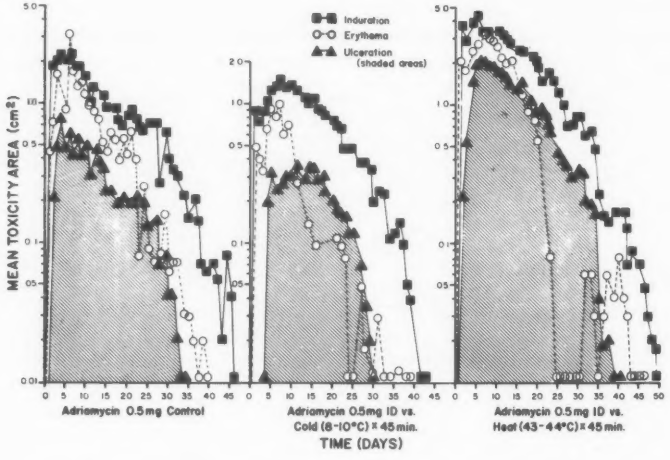

FIGURE 2.—Semilogarithmic plots of mean skin lesion areas (cm^2) vs time (days) for animals (n = 5 each) receiving 0.5 mg of intradermal (ID) DOX (control, left graph); 0.5 mg of intradermal DOX + 45 mins of topical cold, 8°C–10°C, applied immediately (middle graph); and 0.5 mg of intradermal DOX + 45 mins of topical heat, 43°C–44°C, applied immediately (right graph).

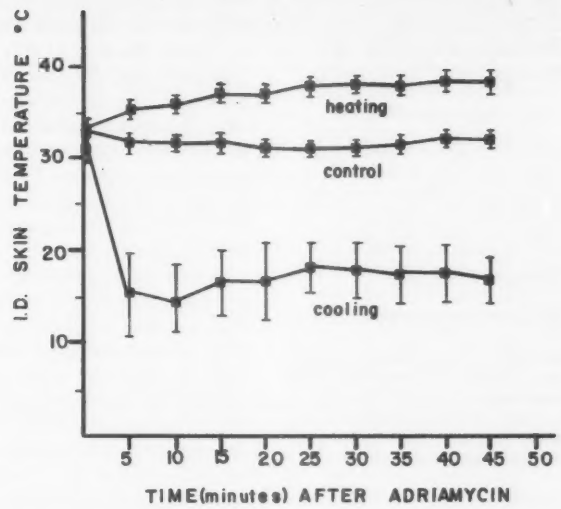

FIGURE 3.—Intradermal (ID) skin temperature changes in mice (n = 3 each) following topical heating, 43°C–44°C, for 45 mins (top line); no therapy (control, middle line); and cooling, 8°C–10°C, for 45 mins (bottom line).

tions and increased plasma concentrations 1–2 hours after dosing in cooled animals. This trend was not maintained over time and there were no significant differences in DOX skin and plasma levels 2 hours after DOX injection.

The in vitro clonogenic assay studies demonstrated significantly reduced DOX effects at temperatures < 37°C (fig 5). There was no DOX-induced cytotoxicity at 4°C. At 20°C, 10 μg/ml for 1 hour reduced survival by only 50% as compared to 5% survival at 37°C and 41.8°C. These data clearly show that cold exposure reduces DOX cytotoxicity in vitro.

DISCUSSION

The results of this study show that DOX skin toxicity in the mouse is reduced significantly by topical cooling. In addition, heating caused duration-dependent early DOX lethality following otherwise nonlethal intradermal doses of 0.05 mg (7 mg/m^2) and 0.5 mg (70 mg/m^2). This

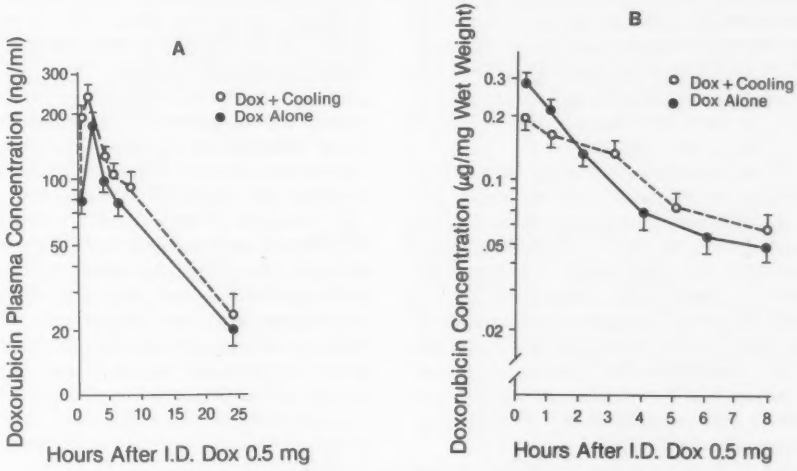

FIGURE 4.—DOX disposition in plasma (A) and skin (B) as determined by HPLC studies.

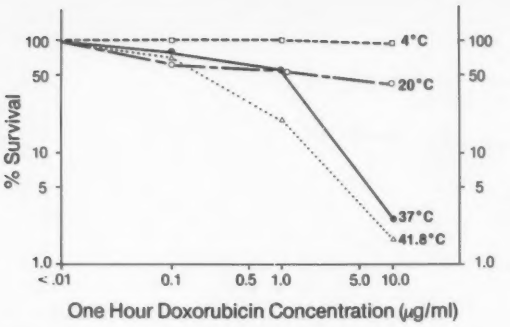

FIGURE 5.—% survival of clonogenic HEC-1A human endometrial carcinoma cells exposed to DOX for 1 hr at different temperatures. Plating efficiency (No. of colonies/No. of cells plated/dish × 100) ranged from 0.003 to 0.006 or 100–200 colonies/dish. Both survival and drug concentration are plotted logarithmically. Each point represents the mean of triplicate experiments.

observation on survival, however, is based on very few animals. Cooling was consistently lethal to 20% of the animals in each mouse group, while up to 80% of animals treated with heat and high-dose intradermal DOX died. Because of the substantial lethality with 60 minutes of heating after DOX, other toxicity parameters including AUC peak lesion size and damage duration appear artificially low in these treatment groups (table 1).

Pentobarbital anesthesia was abandoned in this study to assess the ability to topically heat or cool mice with intact thermoregulatory mechanisms. Pentobarbital is known to alter regional blood flows and to partially ablate normal thermoregulatory mechanisms in the mouse (11).

The toxicity reductions obtained with cooling were apparent following both intradermal DOX doses (0.05 and 0.5 mg). These results are substantially better than those following pharmacologic manipulations using glucocorticosteroids (13) or beta adrenergic agents (14). Thus, statistically significant protective effects from these pharmacologic antidotes were observed only after the lower intradermal DOX dose of 0.05 mg.

The enhanced skin toxicity and early lethality of DOX plus hyperthermia are consistent with numerous previous reports of DOX-hyperthermia synergism (12–17). Besides DOX, a number of other antineoplastic drugs have also shown experimental synergism with hyperthermia. These include cisplatin (18), mitomycin (19), the nitrosoureas carmustine and lomustine (20), methotrexate (21), thiotepa (22), mechlorethamine (23), cyclophosphamide (23), and melphalan (24). Conversely, general *hypothermia* has been used with some success as a systemic antidote to regional applications of melphalan, mechlorethamine, and 5-FU (25,26).

The mechanism of the toxic enhancement for heat and DOX seen in this study and in others remains speculative. Both Block et al (27) and Hahn and Stravde (13) have previously demonstrated that cell culture temperatures > 37°C greatly enhance the cellular uptake of DOX. Thus, the cellular influx of DOX would appear to be temperature-dependent, with uptake significantly enhanced by heat and decreased by the cooler temperatures. This may be the primary mechanism for the cold protection of mouse skin seen in the current study. The in vitro clonogenic assays also support this mechanistic hypothesis.

Besides augmenting some drug effects, hyperthermia also has direct effects on cell populations. Early classic studies of heat effects on normal cell cycle and growth kinetics showed that hyperthermia stimulates nonproliferating bone marrow stem cells into a proliferative (and therefore more chemosensitive) state (28–30). Thus, in this study, topical heating may have similarly enhanced the growth fraction in the skin, thereby increasing apparent DOX effects. As a biologic corollary, local heating also synergizes with radiation, increasing tumor cell kill, while cold can protect tissues from radiation damage (31). Since DOX is known to synergize with radiation, there may be important similarities in the mechanism of action of these different treatment modalities.

We were not able to demonstrate significantly altered DOX disposition in skin or plasma following cooling. In contrast, Skibba et al (32) have demonstrated enhanced DOX retention in perfused rat livers at elevated temperatures.

In patients with sarcoma treated by Riggs et al (33), whole-body hyperthermia slightly increased both DOX plasma concentration-time products (determined by total anthracycline fluorescence), and the amount of the active alcoholic metabolite, adriamycinol (determined by HPLC). Mimnaugh et al (34) also evaluated DOX disposition and metabolism when combined with whole-body hyperthermia in rabbits. There were no significant differences in DOX elimination rates between heated and unheated animals in this study. These latter observations are similar to our results in the mouse skin model. However, a major limitation of our methodology involved the use of hemogenates of whole skin tissue which did not discriminate between intracellular and extracullular (interstitial) DOX within the local skin site.

In summary, it appears that a duration of at least 45–60 minutes of cooling after DOX extravasation is necessary to achieve maximal benefit. Since the skin cooling of the mice in this study was very efficient, the use of a longer duration but less efficient skin cooling in patients might achieve the same level of protection. It is not advisable to aggressively reduce human skin temperatures since hypothermic injuries could occur. The use of topical hyperthermia for DOX clinical infiltrates should be abandoned completely due to the toxic enhancement seen in this study.

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Allergic Reactions to Teniposide in Patients With Neuroblastoma and Lymphoid Malignancies[1,2]

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Acute allergic reactions have been reported in 1%–5% of patients receiving teniposide. In a patient population of 82 children with leukemia and lymphoma, we observed only two acute reactions during the infusion of 1707 doses of teniposide. In contrast, 14 of 105 children with neuroblastoma developed acute allergic reactions during the infusion of 665 doses of teniposide. Four rechallenge doses of the drug were given to patients with neuroblastoma after premedication with antiallergic agents. All four doses resulted in repeat reactions. Our data suggest that children with neuroblastoma have a significantly higher incidence of acute reactions to teniposide than patients with other malignancies ($P = 0.008$), and that these reactions cannot be prevented by premedication with antiallergic drugs. [Cancer Treat Rep 69:439–441, 1985]

Allergic reactions to drugs may be manifested as acute anaphylactic reactions, with cardiovascular and pulmonary instability and rapidly developing edema and urticaria, or as delayed reactions usually limited to skin rashes and fever. Some allergies appear to require prior exposure to the agent or similar agents, while others occur without prior exposure.

Over the last 7 years, teniposide has been used in phase II and III trials in children at St. Jude Children's Research Hospital. We have observed a marked difference in the incidence of acute allergic reactions occurring in patients with neuroblastoma compared to patients with other malignancies.

PATIENT POPULATION

As part of phase II and III trials, 105 children with neuroblastoma received 665 infusions of teniposide. The number of doses delivered ranged from one to 24 per patient and the dose per infusion was, in almost all cases, 100 mg/m^2 infused over 30–45 minutes.

Eighty-two children with high-risk acute lymphoblastic leukemia or lymphoma received 1707 infusions of teniposide. The number of doses delivered ranged from one to 28 per patient, and the dose per infusion was 165 mg/m^2.

Statistics were performed using Fisher's exact test.

RESULTS

Of the 105 patients with neuroblastoma, 14 (13%) had acute allergic reactions to teniposide. Four patients were rechallenged after premedication with Benadryl, and all had repeat acute allergic reactions. Thus, 18 acute reactions occurred during 665 infusions of teniposide in children with neuroblastoma. These reactions occurred within minutes of starting the infusion, with rapid onset of symptoms such as flushing, urticaria, wheezing, coughing, and vomiting (table 1). Acute rises in blood pressure occurred in six patients, while one had severe hypotension and required constant infusion of dopamine for 24 hours before the blood pressure stabilized. Except for this latter patient, all other reactions were controlled by iv Benadryl and Solu-Cortef.

In contrast to the acute reactions seen in 14 of 105 children with neuroblastoma, only two acute allergic reactions were observed in 82 children (2.8%) receiving this drug as part of therapy for high-risk acute lymphoblastic leukemia or lymphoma ($P = 0.008$). These children were not receiving steroid therapy concomitantly. These two acute reactions occurred during the infusion of 1707 doses of teniposide in these patients, compared to 18 acute reactions occurring during 665 infusions in the children with neuroblastoma ($P < 0.001$).

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TABLE 1.—Manifestations of hypersensitivity to teniposide in 14 patients with neuroblastoma

Patient No.	Teniposide Course*	Edema	Urticaria, pruritis	Flushing	Wheezing cough, cyanosis (c)	Vomiting	Hypertension†	Hypotension
1	1		+	+	+	+	+	
2	1		+	+	+			+‡
3	9	+	+	+	+	+	+	
4	6		+	+	+			
5	3		+	+			+	
6	5		+	+				
7	2		+	+	+(c)	+	+	
8	10		+	+	+	+		
9	2	+	+	+	+			
10	6		+	+		+	+	
11	1	+	+	+	+	+	+	
12	6	+	+	+	+	+		
13	2	+	+	+	+	+		
14	2				+(c)			

* Course with which first reaction occurred.

†Resolved when reaction was controlled with Benadryl and Solu-Cortef.

‡Dopamine drip for 24 hrs before blood pressure stabilized.

Delayed reactions occurred in three children with neuroblastoma, with chills, rash, or hypotension occurring within 3 hours of drug administration. These three patients all received further teniposide, with only one having a second delayed allergic reaction. In the children with leukemia or lymphoma, delayed reactions with fever, rash, and/or hypotension occurred in 12 patients, two of which were associated with prolonged and severe hypotension. When premedicated with antiallergic drugs, all 12 of these patients received subsequent teniposide without incident.

The acute reactions in the children with neuroblastoma were unpredictable, occurring with the first dose of teniposide in three patients, with the second dose in four, and with the third to tenth course in the remainder (median, six).

Reactions to etoposide, another semisynthetic podophyllotoxin, were not observed in the five patients with neuroblastoma who received this agent without premedication with antiallergic drugs after having acute allergic reactions to teniposide.

DISCUSSION

Descriptions of acute allergic reactions to teniposide have been reported infrequently. In a review article by Weiss and Bruno (1), an incidence of 1%–2% was reported. Bleyer et al (2) reported one anaphylactic reaction in a group of 116 patients, which included 39 children with neuroblastoma, while Mathé et al (3) reported two anaphylactic reactions in 56 patients being treated for lymphoma or leukemia.

The mechanism by which teniposide induces allergic reactions is not known. It has been shown in dogs that iv teniposide results in a significant increase in the blood histamine levels, which correlated with reduction of cardiac inotropism. These effects could be ameliorated by pretreatment with antihistamines (4). The same cardiac effects induced in animals by teniposide could, to a lesser degree, be induced by the injection of the commercial solvent[4] used for preparation of the drug for injection. It is a possibility that the reactions seen with teniposide are due to the solvent and not the drug. Both diaziquone and formulated amsacrine have dimethylacetamide as a solvent, and both have been reported as causing allergic reactions (5,6). Cremophor EL has also been implicated in the anaphylactic reactions occurring with agents using it as a solvent (7,8). Etoposide, a closely related compound to teniposide, does not require this solvent for preparation, and no acute anaphylactic reactions have been ascribed to this drug.

Another possible mechanism would be the development of the antibody to teniposide. One patient has been described with an IgG_1 antibody to teniposide (9). This patient had previously had transient pruritis, rash, and thrombocytopenia associated with infusions of this drug. Habibi et al (9) reported four other patients with allergic reactions to teniposide in whom no antibody could be demonstrated.

There is no obvious reason why the occurrence of acute reactions should be so frequent in our patients with neuroblastoma. One-half of the reactions seen occurred after multiple exposures to the agent, and it is possible that patients with neuroblastoma entered into Bleyer's (2) trial received too few doses of teniposide for the higher incidence of reactions to occur. In our neuroblastoma population, two of 29 patients (7%) receiving teniposide

[4]Contained dimethylacetamide (300 mg), Cremophor EL (2.5 g), and ethanol (5 ml/50 mg of teniposide).

as a single agent reacted, compared with 12 of 76 (16%) who were given the drug 48 hours following cisplatin ($P = 0.34$).

Regardless of the mechanism, it is important that the high incidence of acute allergic reactions to teniposide in patients being treated for neuroblastoma be recognized. Unlike patients with leukemia and lymphoma who more frequently have delayed reactions, premedication with antiallergic drugs has not prevented these acute allergic reactions from occurring.

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Doxorubicin, Dacarbazine, Vincristine, and Cyclophosphamide in the Treatment of Advanced Gastrointestinal Leiomyosarcoma[1]

T. K. Choi,* A. Ng, and J. Wong[2]

A multidrug regimen of doxorubicin, dacarbazine, vincristine, and cyclophosphamide produced response in 33% of patients with advanced gastrointestinal leiomyosarcoma. Except for one patient, the duration of response was short and prolongation of survival was not affected. [Cancer Treat Rep 69:443–444, 1985]

Leiomyosarcoma is a malignant neoplasm arising from smooth muscles and has been reported to occur in virtually all organs in the body containing smooth muscle cells. Surgical resection is the treatment of choice when the tumors are localized. However, a significant proportion of patients have unresectable tumors at the time of presentation, and the metastatic and local recurrence rate is high following resection. Only 40% of patients with gastric (1), 25%–40% of small intestinal (2,3), and 24%–35% of colorectal leiomyosarcomas (3,4) have survived > 5 years after diagnosis. Most patients died with widespread metastasis to the liver, retroperitoneum, lung, and lymph nodes. An effective systemic form of treatment is needed to prolong the survival of these patients.

In this article, we report the results of treatment of 12 patients with advanced gastrointestinal leiomyosarcoma using doxorubicin, dacarbazine, vincristine, and cyclophosphamide. This combination of drugs was chosen because of the 50%–59% response rate obtained in several series of patients with sarcomas (5–7).

PATIENTS AND METHODS

This trial was prospectively conducted. The selection criteria were as follows: (*a*) histologically proven leiomyosarcoma of the gastrointestinal tract not resectable because of extensive local infiltration, metastasis, or recurrence after resection; (*b*) the presence of a measurable lesion; (*c*) a Karnofsky rating of ⩾ 40%; (*d*) an initial wbc count of > 5000/mm^3 and a platelet count of > 100,000/mm^3; (*e*) no ECG abnormalities and no previous history of heart disease; (*f*) no previous chemotherapy; and (*g*) patient consent for chemotherapy. Between January 1978 and December 1983, 13 patients satisfied the above criteria and were entered in the trial, but one withdrew after receiving one course, leaving 12 evaluable patients. The drugs, which consisted of doxorubicin (60 mg/m^2), vincristine (1 mg/m^2), and cyclophosphamide (200 mg/m^2) on Day 1, and dacarbazine (200 mg/m^2) on Days 1–3, were administered iv every 3 weeks. Prochlorperazine (12.5 mg) was given im before each drug injection to combat nausea. A maximum of ten courses was given. The size of the measurable lesions, a cbc with platelet count, and an ECG were recorded before the administration of each course. If the wbc count was < 3000/mm^3 or the platelet count was < 100,000/mm^3, the dose of drugs was halved. If the wbc count was < 2000/mm^3 or the platelet count was < 70,000/mm^3, chemotherapy was stopped for 3 weeks. Chemotherapy was terminated if the tumor increased in size, if there was no response after four courses of treatment, if the Karnofsky rating dropped below the 40th percentile, or if there were any significant changes in the ECG. Complete response was defined as the absence of clinically detectable tumor; partial response was defined as tumor shrinkage > 50%, or when the sum of the liver span between the liver edge and the right costal margin at the midclavicular line and the xyphoid decreased by more than one-third. No response was defined as a lesser degree of response, stabilization, or increase in the size of the tumor.

RESULTS

There were six men and six women in the study. The youngest patient was 24 years of age and the oldest was 73, with a mean age of 52. The primary tumor was lo-

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cated in the stomach in five patients, in the duodenum in three, in the jejunum and ileum in three, and in the rectum in one. Five patients had excision of the tumor performed 1-9 years before recurrence was detected. All patients had metastases to the liver, two had peritoneal carcinomatosis, and one had lung metastases. The patients received a mean of 5.7 courses of chemotherapy, with a range of two to ten courses.

Two patients had complete responses and two had partial responses, giving an objective response rate of 33.3%. The duration of response for the two complete responders was 6 and 36+ months; the duration for the partial responders was 3 and 7 months. The survival for the responders was 4, 10, 15, and 38+ months; the survival for the nonresponders was 2, 4, 4+, 5+, 6, 7, 15, and 25 months. Except for one patient who died of liver failure, the cause of death in all patients was terminal cachexia or complications related to the tumor.

One of the complete responders was a 24-year-old woman who underwent an exploratory laparotomy for attempted excision of a gastric leiomyosarcoma, but was found to have an unresectable tumor because of retroperitoneal infiltration and extensive lymph node and liver metastases. After two courses of drugs, she achieved a partial response and her general condition improved dramatically. No tumor was clinically detectable after four courses, and she has remained free of tumor for 38 months after the start of chemotherapy.

In one 60-year-old woman with a partial response, the liver shrank dramatically in size after two courses, but her condition deteriorated with the development of ascites and liver failure. She also had marked leukopenia, and drug administration had to be stopped after four courses. She eventually died of liver failure.

Except for the patient with a partial response mentioned above, the side effects were generally mild and tolerable. Leukopenia < 5000 cells/mm^3 occurred in eight patients, three of whom had leukopenia < 3000 cells/mm^3. Other toxic effects included alopecia (83%) and nausea and vomiting (67%).

DISCUSSION

The most effective drugs in the treatment of advanced sarcomas are probably multidrug regimens containing doxorubicin and dacarbazine. The response rate has been reported to be between 11% and 59% (5-10), with most reports around 50%. Because of the relatively low incidence of sarcomas, the number of tumors in any series with the same histological type and primary site was small. The relationship between these two factors and the rate of response has not been adequately studied. Our results for the treatment of gastrointestinal leiomyosarcomas were slightly lower than those reported for sarcomas treated by the same drugs. Metastatic sarcomas have been found to be less responsive to chemotherapy than local sarcomas (10). All of the patients in this study had advanced metastatic tumors, which may account for the lower response rate seen. Another cause for the lower response rate may be the slightly lower dosage of drugs used in this trial.

Subramanian and Wiltshaw (10) found that the complete responders tended to be young females (< 30 years of age). Our only patient who benefited, a 24-year-old female, further illustrates this important feature. This patient is free of tumor at 38 months after the start of chemotherapy. The duration of remission of the other three responders was short. These responders died soon after the relapse of tumor growth, so that their survival was not prolonged when compared with the nonresponders.

In conclusion, the combination of drugs used in this study has activity against gastrointestinal leiomyosarcomas. However, the proportion of patients benefited is small. For more effective treatment, it would be necessary to evaluate other drugs or combinations of drugs.

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Treatment of Chronic Myeloid Leukemia Blast Crisis With Vindesine and Prednisone[1]

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Sixteen patients in chronic myeloid leukemia blast crisis entered a phase II trial with vindesine and prednisone. Median duration of the chronic phase was 29 months in 13 patients, one previously had polycythemia vera, and two presented with a primary blast crisis. Eleven patients had myeloblastic features, as evidenced by morphology, cytochemistry, and cell surface antigens; three had a mixture of myeloid and lymphoid blast cells with lymphoblastic predominance; one had blast cells which displayed lymphoid characteristics; and one was classified as undifferentiated. Three patients had complete remissions lasting 1 month (myeloid), 3 months (mixed), and 5 months (lymphoid). Eleven patients had minor responses, with a median duration of 3 weeks (eight with myeloid, two with mixed, and one with undifferentiated). Two patients did not respond to vindesine. Leukopenia and thrombocytopenia were severe and prolonged independent of their morphologic or immunologic phenotype. [Cancer Treat Rep 69:445–448, 1985]

In spite of substantial progress in antineoplastic chemotherapy and supportive care, the treatment of blast crisis in chronic myeloid leukemia (CML) remains a major problem. In 1971, it was noted that about one-third of patients with CML blast crisis responded to vincristine and prednisone (1), agents which are often effective in the treatment of acute lymphoblastic leukemia but not myeloid leukemia. Some studies have shown a correlation between responsiveness to this type of treatment and the presence of lymphoid leukemic cells in blast crisis (2–4); other studies have failed to show this correlation (5,6). Vindesine (VDS), a more recently developed semisynthetic vinca alkaloid, has been reported to be more effective in some hematologic malignancies, but, due to its pharmacokinetic properties, less toxic than vincristine and vinblastine (7,8). The purpose of this study was to see whether the favorable effects of VDS, observed in small series of patients with CML blast crisis (9,10), could be confirmed in a larger cohort, and whether the sensitivity of blast cells to this drug is associated with their lymphoid or myeloid nature.

PATIENTS AND METHODS

Sixteen consecutive patients entered the study. Their clinical characteristics are listed in table 1. The median age of the patients was 46 years; the median duration of the preceding chronic phase was 29 months in 13 patients. One patient (No. 13) developed CML blast crisis after polycythemia vera lasting 6 years; two others (Nos. 2 and 4) had a primary blast crisis without an antecedent chronic phase. Twelve patients had received busulfan during their chronic phase; four received 6-mercaptopurine and 3-hydroxyurea in addition. X-irradiation of the spleen was performed on two patients.

Treatment protocol.—VDS (2 mg/m^2) was given weekly by bolus injection on 2 consecutive days for at least one cycle (mean, 2.7), with a total dose of 3–34 mg (mean, 16.7). The duration of treatment was adjusted according to platelet and leukocyte counts. During the first week, prednisolone was administered orally at 75 mg/day, during the second week at 50 mg/day, and during the following weeks at 25 mg/day.

Blast crisis.—This was defined as increasing blast counts and promyelocytes in the peripheral blood > 30%, hemoglobin < 10 g/dl, and unexplained fever lasting 5 days. Response was assessed by regular clinical examination and laboratory investigations, including bone marrow aspiration when blast cells disappeared from the peripheral blood. Complete remission (CR) was defined as the disappearance of all clinical symptoms, and normalization of the blood cell counts (no blasts or promyelo-

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TABLE 1.—Clinical characteristics

Patient No.	Age (yrs)	Sex	Duration of chronic phase (mos)	Type of pretreatment*	Cytogenetics	Cytochemistry†	Membrane markers	Morphology‡	Bone marrow histology (myelofibrosis)	Alkaline phosphatase§	Basophils (% peripheral blood)
1	70	F	39	Busulfan, 6-MP, ^{32}P, mitobronitol	ND¶	ND	73% myeloid 1:64	M3	ND	1	17
2	68	F	0	None	Ph1-negative 47,xx,+13	POX II a phos-positive NAS-positive	24% myeloid 1:64	M2	ND	Negative	1
3	31	M	10	Busulfan, RT to spleen, splenectomy	Ph1-positive 46,xy,iso 17q	ND	60% myeloid 1:64	M3	Yes	36	25
4	27	F	0	None	Ph1-positive 46,xx	POX-negative a phos-positive	30% myeloid 70% cALLA	L2	No	6	1
5	17	F	10	Busulfan, 6-MP	Ph1-positive 46,xx	ND	34% myeloid 1:128	M4	No	Negative	3
6	46	M	9	Busulfan, hydroxyurea	ND	ND	ND	M3	No	ND	22
7	39	M	48	Busulfan	Ph1-positive 54,xxyy	POX-negative a phos-positive PAS-positive	90% cALLA 1:256	L2	No	34	2
8	43	M	20	Busulfan, chlorambucil	ND	POX-negative PAS-negative	50% myeloid 1:32	M2	No	Negative	30
9	52	F	20	None	Ph1-negative 46,xx	POX II PAS-negative a phos-positive	40% myeloid 1:32	M2	No	Negative	1
10	34	M	2	Busulfan	Ph1-positive	POX-negative	32% myeloid 55% cALLA	L2/M2	Yes	Negative	7
11	54	M	40	Busulfan, 6-MP, RT to spleen	Ph1-positive 46,xy	ND	ND	M2	Yes	Negative	8
12	25	F	31	Busulfan, 6-MP	ND	POX-negative PAS-negative	None (undifferentiated)	M0	Yes	76	20
13	54	M	66	^{32}P	ND	POX-negative PAS-negative a phos-positive	90% T28, LYT3 1:512 80% myeloid 1:128	L2/M1 (Auer rods)	No	Negative	1
14	63	F	43	Busulfan, hydroxyurea	ND	ND	40% myeloid 1:16	M2	ND	ND	40
15	50	F	68	Busulfan, hydroxyurea, RT to spleen	ND	ND	51% myeloid	M3	No	Negative	3
16	60	F	36	Busulfan	ND	POX-negative PAS-negative	70% myeloid 1:32	M2	Yes	7	0

*6-MP = 6-mercaptopurine; RT = radiation therapy.
†POX = peroxidase; a phos = acid phosphatase.
‡French-American-British classification.
§Normal range of index = 10-100.
¶ND = not determined.

cytes in the differential count, < 5% blasts in the bone marrow). Minor response was defined as > 50% reduction of the absolute blast and promyelocyte counts in the peripheral blood, and stabilization of all blood values without the need for substituting blood components. Cytochemical stainings of bone marrow smears for evaluation of PAS, peroxidase, nonspecific esterase, and acid phosphatase were performed in nine of 16 patients. Multimarker analysis using immunologic methods was performed in 14 of 16 patients by Dr. Thiel from the GSF-Institute for Hematology, Munich. Cytogenetic studies were carried out in eight patients by Drs. Baumann and Clemm from our institution.

RESULTS AND DISCUSSION

By morphological, immunological, and cytochemical criteria, 11 patients displayed myeloid-like blast cells, one displayed lymphoid-like blast cells, three showed a mixture of myeloid and lymphoid cells with lymphoblastic predominance, and one was undifferentiated, including a negative staining for terminal deoxynucleotidyltransferase (table 10). All but one of the patients had either excessive hepatosplenomegaly (six) or splenomegaly (nine) at the time of blast crisis. Five of 13 patients showed myelofibrosis upon histological examination. Six of eight patients were found to have a Philadelphia chromosome and two were Ph1-negative. Six patients had other or additional chromosome aberrations.

Treatment results are summarized in table 2. CR was achieved in three patients with myeloid (No. 1), mixed (No. 4), and lymphoid (No. 7) differentiation. Remissions were of 1, 3, and 5 months' duration, respectively. Patient 7 experienced a second CR lasting 6 months using the same treatment protocol. One of the responders (No. 4) had a primary blast crisis without a preceding chronic phase, which might be termed Ph1-positive acute leukemia (known to respond to chemotherapy). However, it is noteworthy that the blasts were phenotypically mixed, suggesting, together with high platelet counts at diagnosis, an activated myeloproliferative syndrome rather than acute lymphoblastic leukemia. Interestingly, the patient's sister also suffered from Ph1-positive CML in chronic phase. In 11 patients, a minor response was obtained with disease stabilization of short duration (1–8 weeks; mean, 3 weeks). One of these patients (No. 10), with a mixed blast cell population, was in his second blast crisis and had experienced a previous CR after treatment with vincristine/prednisolone. Five of these patients had a marked regression (> 50%) of their spleen size (sp+) or hepatosplenomegaly (sp+/l+). Eight patients with minor response had a myeloid differentiation, two were mixed, and one was undifferentiated. By definition, all patients with minor response showed a > 50% reduction of absolute blast cell counts; in addition, eight showed an improvement of their differential count. Two patients with myeloid-type blasts did not respond.

TABLE 2.—Treatment results

Patient No.	Duration of VDS (wks)	Total dose of VDS (mg)	Response*	Duration of response (wks)	50% Reduction of spleen/liver size	Wbc count (10^9/L)†		Platelet count (10^9/L)†		% blasts				Side effects of VDS
										Bone marrow		Peripheral blood		
						Before	After	Before	After	Before	After	Before	After	
1	2	12	CR	4	sp+	266	8.4	390	227	ND	4	59	0	—
2	2	11	PD		No enlargement	7.8	3	65	40	62	71	33	22	—
3	2	9.7	MR	2	sp-/l-	113	6.1	20	23	ND		39	5	Paresthesia
4	5	34	CR	14	sp+	133	21.6	233	222	66	4	68	0	Paresthesia, loss of reflexes
5	4	26.6	MR	2	sp-/l-	148	21	57	62	ND	23	69	82	—
6	2	10.5	PD		sp-	150	18	25	15	58		35	27	—
7	3	21.6	CR	21	sp+	13.9	2	23	223	83	1	85	0	Paresthesia
8	2	12.2	MR	1	sp+	48.9	8	412	80	ND		38	64	—
9	5	24	MR	8	sp-/l-	207	74	150	150	54	ND	26	19	Paresthesia
10	2	14	MR	1	sp-	30.3	4.6	35	49	ND		52	15	Paresthesia
11	2	14.4	MR	3	sp-/l-	53.8	2.8	167	180	25	ND	35	3	—
12	3	16.8	MR	3	sp+	5.5	1.7	101	10	70	ND	37	2	Paresthesia
13	3	30.4	MR	4	sp+	81	10	57	10	90	84	94	49	—
14	2	9.6	MR	3	sp-	16.2	2.8	34	47	ND		25	27	—
15	½	3	MR	1	sp+/l+	97	1.2	14	60	ND	34	66	0	—
16	3	18	MR	2‡	sp+/l+	34.7	6.8	632	255	60	ND	41	13	—

*PD = progressive disease; MR = minor response. Patient 7 had a 2nd CR with the same treatment. Patient 10 had a previous CR with vincristine/prednisolone.
†Values for wbc and platelet counts after treatment refer to those of disease stabilization, not to the leukocyte or platelet nadir.
‡Patient 16 achieved MR repeatedly, surviving 10+ mos.

The ablative capacity of this regimen was quite pronounced, irrespective of a myeloid or lymphoid-like blast cell differentiation which was accompanied by a good relief of bone or spleen pain and an impressive regression in organomegaly in most cases. The wbc count nadir occurred within 12–13 days. Thrombocytopenia was equally severe, requiring heavy platelet support. Both patients with progressive disease (Nos. 4 and 6) showed either an increase or only minor (less than a log) reduction of wbc count. Neurological side effects, mainly consisting of paresthesia, occurred in six of 16 patients and were, with one exception (No. 4), mild.

As judged from the duration of CRs and the repeated responses observed in the same patients (Nos. 7 and 10), it appears that lymphoid-like differentiation might be more favorable than myeloid. Still, the number of patients studied is too small to preferentially associate the effect of treatment with one or the other blast cell type. The short duration of minor response is unsatisfying. The marked reduction of tumor load in these patients, however, should be utilized to continue with another kind of treatment for maintenance as soon as a stabilization of clinical symptoms and the blood cell counts has been reached. This could possibly delay or inhibit further proliferation of the malignant cell clone after cessation of VDS effect. In two recent cases, we successfully maintained minor responses with 6-mercaptopurine. Maintenance treatment with VDS has its limitations because of neurological toxic effects, which become more prominent as time increases. Individual dose and schedule modifications during induction therapy and combination with other drugs for early maintenance treatment should be attempted in future trials.

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Cisplatin in the Treatment of Advanced Gastric Carcinoma: A Phase II Study[1]

Kristian Aabo,* Hans Pedersen, and Mikael Rørth[2]

Twenty-one patients with advanced local or metastatic gastric carcinoma were treated with 75 mg/m² of cisplatin every 3 weeks. Six patients had received no prior chemotherapy. One patient achieved complete tumor regression lasting 5 months, while three patients had partial responses of 2, 4, and 11 months' duration, respectively, resulting in an overall response rate of 18% (95% confidence limits: 5%–40%). All four responding patients had improvement in performance status. Two of six patients who had had no prior chemotherapy responded, while only two of 16 patients previously treated with 5-FU and doxorubicin responded. [Cancer Treat Rep 69:449–450, 1985]

During the last decade, cisplatin has shown beneficial therapeutic effects in testicular and ovarian cancers and is now accepted as an important part of combination chemotherapy regimens for these diseases. The present study was undertaken to define the activity of cisplatin in gastric cancer.

MATERIAL AND METHODS

Patients with histologically confirmed, evaluable, and progressive gastric carcinoma were eligible for study. Evaluable disease was defined as: (*a*) palpable abdominal or pelvic masses and/or abdominal masses clearly documented by ultrasound; (*b*) hepatomegaly palpable > 5 cm below the costal margin in the right midclavicular line owing to liver metastases verified by ultrasound; (*c*) neck lymph node and cutaneous metastases; or (*d*) metastatic lung lesions demonstrated on chest x-ray.

Pretreatment evaluation included physical and pelvic or rectal examinations; chest x-ray; abdominal ultrasound; roentgenological examination of the upper gastrointestinal tract; audiograms; chrome-EDTA renal clearance; and blood tests including serum creatinine, serum CEA, SGOT, serum alkaline phosphatase, hemoglobin, platelet count, and wbc count. Except for ultrasound and upper gastrointestinal x-ray examination, which were repeated in relation to every two treatments, the examinations were repeated prior to each treatment. Time from previous chemotherapy had to be at least 4 weeks. The age of the patient was required to be ⩽ 75 years, and the expected survival had to be > 4 weeks. Normal kidney function evidenced by normal chrome-EDTA clearance was mandatory. Informed consent was given by all patients.

Response was defined according to the World Health Organization recommendations. In the case of hepatomegaly, the reduction in liver size (> 50% of the extension below the costal margin) had to be concomitant with improvement in blood tests of liver function, measured as decrease in serum alkaline phosphatase.

Cisplatin at a dose of 75 mg/m² in 500 ml of 10% mannitol was given iv over 1 hour every 3 weeks. If the patient's tolerance was adequate, the dose was increased to 100 mg/m². Pretreatment hydration was given orally with at least 2½ L of fluid starting the night before. Before cisplatin administration, 1 L of glucose/saline plus 500 ml of 10% mannitol was given. After cisplatin administration, 2 L of glucose/saline was administered iv. The characteristics of the patients are shown in table 1.

RESULTS

Among the 22 patients included in the study, ten completed protocol treatment on an outpatient basis, while the remaining 12 patients required 2–3 days' hospitalization in relation to the treatments. The median number of treatments was three (range, one to 12). Three patients had dose escalation to 100 mg/m², but one did not tolerate this. Another three patients underwent dose reduction, one owing to disabling neuropathy and two owing to severe emesis.

Four patients achieved partial responses. One patient had complete resolution of cutaneous metastases and disappearance of a palpable epigastric mass but only a

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TABLE 1.—Characteristics of 22 patients with carcinoma of the stomach treated with cisplatin

Characteristics	No. of patients
Median age in yrs (range): 58 (36–75)	
Median performance status (range): 1 (0–3)	
Median time, in mos, from diagnosis to start of cisplatin (range): 10 (½–46)	
Male/female	13/9
Cardia/stomach	7/15
> 10% weight loss prior to treatment	6
Previous chemotherapy*	16
Previous irradiation	0
Evaluable disease	
Metastatic neck lymph nodes	1
Lung metastases on chest x-ray	1
Pelvic mass (pelvic examination + ultrasound)	3
Skin metastases and abdominal mass	1
Abdomen	
Palpable mass (including 1 hepatomegaly)	13
Ultrasound alone	2

* 5-FU + doxorubicin.

70% decrease in serum CEA (from 126 to 45 ng/ml); the response lasted 5 months. Two patients had > 50% reduction in the size of palpable epigastric masses lasting 2 and 11 months. One patient had a reduction in liver size from 7 to 0 cm below the costal margin accompanied by normalization of liver biochemistry and a 95% decrease in serum CEA (from 3330 to 129 ng/ml); the response lasted 4 months. Only the four responders had improvement in performance status. Accordingly, the overall response rate was 18% (95% confidence limits: 5%–40%). The median survival from start of cisplatin treatment was 5 months (range, 0.5–15+).

Concerning toxicity, 15 patients (68%) experienced moderate to severe emesis, including two patients who refused further treatment after one course owing to subjective side effects. In nine patients (41%), hemoglobin was recorded as < 6.5 mmol/L = 10.5 g/100 ml (lowest, 5.1 mmol/L = 8.2 g/100 ml), and three patients (14%) had a decrease in platelet count to < 100 × 10^9/L (lowest, 37 × 10^9/L). None of the patients developed leukopenia. Two patients developed progressive disabling peripheral paresthesias after eight treatment cycles. In one of these patients cisplatin was discontinued because of this complication after 12 treatment cycles while the patient was still in partial remission. Since only four patients received eight or more treatments, this complication had a high prevalence (50%) using the present dose schedule if multiple treatments were administered. Four patients had transient increase in serum creatinine, but only two of these patients (9%) developed moderate decrease in chrome-EDTA clearance.

DISCUSSION

Gastric cancer has a grave prognosis, with an overall 5-year survival rate of 7% (1). Although response rates varying from 7% to 26% have been reported for several cytotoxic agents, there has been little impact on survival using single agents (2).

The urgent need for new and more active drugs has, during the last 3–4 years, directed interest towards cisplatin, since this drug has been shown to be important in the treatment of other solid tumors. In recent published studies on the effect of cisplatin in gastric cancer, two complete and ten partial remissions among 74 evaluable patients were reported, an activity similar to our results (3–7). The response rate in these studies varied from 6% (7) to 33% (5). Generally, the response durations were short.

It is concluded that cisplatin is active in gastric cancer; however, its activity as a single agent in the primary treatment of gastric cancer has yet to be defined, as only a few patients treated with cisplatin in phase II trials have not been previously treated. Preliminary studies suggest that cisplatin in combination with 5-FU and doxorubicin may induce significant responses in 50% of the patients (8,9), results comparable with those achieved with the combination of 5-FU, doxorubicin, and mitomycin (10).

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Phase II Study of Aclarubicin and Diaziquone in the Treatment of Advanced Small Cell Bronchogenic Carcinoma (EST 4581): An Eastern Cooperative Oncology Group Study[1,2]

Martin D. Abeloff,* Dianne M. Finkelstein, Alex Yuang-Chi Chang, Fernando J. Camacho, Richard H. Creech, and David S. Ettinger[2]

This randomized phase II study was designed to evaluate the therapeutic efficacy of aclarubicin (ACM-A) and diaziquone (AZQ) in patients with histologically confirmed advanced small cell lung cancer (SCLC). ACM-A (1) is a new anthracycline antibiotic which has not caused alopecia or significant cardiac toxicity in prior trials, and AZQ (2) is a presumed alkylating agent with lipid solubility and ionization characteristics resulting in good penetration of the CNS.

MATERIALS AND METHODS

Sixty-six patients entered this study between March 1982 and July 1983. Fifty-two patients were evaluable for toxicity and response. The 14 unevaluable patients included seven who cancelled prior to starting therapy and seven who were found to be ineligible after treatment had begun.

Patients were eligible for this study if they had measurable or evaluable disease; an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2; and adequate bone marrow, liver, and renal function. Patients must have failed chemotherapy with standard agents and must have had no chemotherapy for at least 3 weeks prior to entry. Patients with brain metastases, prior radiotherapy to present areas of measurable or evaluable disease, prior treatment with doxorubicin > 400 mg/m^2, or history of significant heart disease were excluded. Informed written consent was required. Eligible patients were stratified by performance status (0–1 or 2), extent of disease (limited or extensive), and prior doxorubicin therapy (yes or no).

Treatments were assigned by telephone at a central randomization office. Either 100 mg/m^2 of ACM-A or 40 mg/m^2 of AZQ infused iv over 30 minutes every 3 weeks was given. Patients with performance status of 2 had dose reductions to 80 mg/m^2 of ACM-A and to 30 mg/m^2 of AZQ. Each drug was given until there was clinical evidence of disease progression.

Standard ECOG criteria for solid tumor response and toxicity were used.

RESULTS

The characteristics of the 52 evaluable patients by treatment are shown in table 1. Most patients were male (69%), had extensive disease (92%), had a performance status of 0 or 1 (58%), and had received no prior radiotherapy (58%). A comparison of the treatments indicates that the samples were well-balanced with respect to these on-study variables.

No partial or complete responses were observed in the 52 patients. The median survival (measured from the date of randomization) was 6.7 weeks for patients receiving ACM-A and 8.7 weeks for patients receiving AZQ.

The major toxicity of both drugs was hematologic. Severe or life-threatening hematologic toxicity was documented in 44% of the patients receiving ACM-A and 63% of the patients receiving AZQ. One probable case of AZQ-induced death due to hematologic complications was

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TABLE 1.—Patient characteristics by treatment

Characteristic	No. of patients (%)	
	ACM-A	AZQ
Entered	36	30
Evaluable	25	27
Initial performance status		
0,1	16 (64)	14 (52)
2	9 (36)	13 (48)
Sex		
Male	16 (64)	20 (74)
Female	9 (36)	7 (25)
Age (yrs)		
< 50	4 (16)	1 (4)
50–69	16 (64)	14 (52)
⩾ 70	5 (20)	10 (37)
Unknown	0 (0)	2 (7)
Tumor measurability		
Nonmeasurable	6 (24)	10 (37)
Measurable	19 (76)	17 (63)
Prior weight loss		
None	14 (56)	12 (44)
< 10%	8 (32)	14 (41)
> 10%	3 (12)	4 (15)
Response		
Complete	0 (0)	0 (0)
Partial	0 (0)	0 (0)
No change	3 (12)	1 (4)
Disease progression	22 (88)	26 (96)
Treatment complications		
None	1 (4)	3 (11)
Mild	4 (16)	3 (11)
Moderate	7 (28)	2 (7)
Severe	5 (20)	10 (37)
Life-threatening	8 (32)	8 (30)
Lethal	0 (0)	1 (4)

noted. There was no significant difference between the therapies with respect to the frequency or level of complications.

Because of AZQ's presumed better penetration into the CNS, a comparison was made between the two treatment groups to determine the frequency of progression in the CNS. Among the patients receiving AZQ, one (4%) developed brain metastases, while three (12%) receiving ACM-A developed brain or CNS metastases. This treatment difference was not statistically significant, but sample sizes were too small to detect meaningful differences.

DISCUSSION

In this phase II study, no complete or partial responses were observed in the 52 evaluable patients, but significant hematologic toxicity was documented. It can therefore be concluded with 99% confidence that neither drug was an active agent (ie, at the level of a 20% response rate) in this population of previously treated patients. These results with AZQ are similar to those reported by Fuks et al (3) in their phase II trial of 16 previously treated patients with SCLC. In a broad phase II study of ACM-A, Schutte and colleagues (4) also noted no complete or partial responses in four patients with SCLC and four with non-SCLC.

Therefore, AZQ and ACM-A have demonstrated no activity in previously treated patients with small cell lung cancer, but their efficacy in untreated patients has not yet been tested.

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Cisplatin Neurotoxicity: Failure To Demonstrate Vitamin B_{12} Inactivation[1,2]

Joel Trugman, H. P. C. Hogenkamp, Robert Roelofs, and William J. M. Hrushesky[3,*]

Cisplatin binds in vitro with vitamin B_{12} coenzymes at physiologic pH and temperature. We have hypothesized that cisplatin-induced neurotoxicity is due to an in vivo inactivation of the cobalamin coenzymes. Vitamin B_{12} metabolism was studied in patients treated with cisplatin for advanced cancer. Serum levels of B_{12} and B_{12}-binding capacity remained normal during cisplatin therapy. Excessive urinary excretion of methylmalonic acid and homocystine, which are both metabolic correlates of cellular cobalamin deficiency, was not demonstrated after cisplatin administration. Thus, we find no evidence to implicate cellular B_{12} deficiency as a mechanism of cisplatin neurotoxicity. [Cancer Treat Rep 69:453–455, 1985]

Cisplatin (*cis*-dichlorodiammineplatinum [II]) has toxic effects on neural tissue in vitro and in vivo, including a dose-related peripheral neuropathy (1,2). Although binding to DNA appears to account for much of its antineoplastic effect, the biochemical mechanism underlying cisplatin-induced neurotoxicity remains unknown.

Clinical patterns and experimental data suggest a connection between cisplatin-induced toxicity and vitamin B_{12} deficiency. Sensory neuropathy and progressive anemia are prominent in both conditions. Intracellular vitamin B_{12} is present in two coenzyme forms: methylcobalamin and adenosylcobalamin (fig 1). Methylcobalamin functions as a coenzyme for methionine synthetase (5-methyltetrahydropteroyl-L-glutamate: L-homocysteine 5 methyltransferase), which catalyzes the transfer of the methyl group from 5-methyl-H4-folate to homocysteine. Deoxyadenosylcobalamin is the coenzyme for methylmalonyl-CoA mutase, which catalyzes the interconversion of (L)-methylmalonyl CoA to succinyl CoA. In the absence of an adequate supply of the vitamin, both reactions are limiting, and thus homocystine and methylmalonate accumulate in plasma and urine. Indeed, vitamin B_{12} deficiency is accompanied by a dramatic increase in urinary excretion of methylmalonate from normal trace amounts (0–3.5 mg/24 hours) to as much as 300 mg/24 hours (3). *cis*-Diamminediaquoplatinum (II) has been shown to bind in vitro to both coenzymes at physiologic pH and temperature (4). These cobalamin-diamminediaquoplatinum (II) complexes involve the coordination of the platinum to the lower 5,6 dimethylbenzimidazole ligand in the case of methylcobalamin and to the upper and lower ligands in the case of adenosylcobalamin. These cobalamin-platinum complexes would be expected to be inactive as coenzymes.

On the basis of selective inactivation of the vitamin B_{12} coenzymes, neurotoxicity is not without precedent. Exposure to nitrous oxide causes a myeloneuropathy and megaloblastic anemia clinically similar to vitamin B_{12} deficiency (5); nitrous oxide has recently been shown to inactivate both mammalian cobalamin-dependent enzymes (6). If the neurotoxicity of cisplatin was indeed found to be due to the inactivation of the cobalamin coenzymes, the neurological lesion may be prevented by administering methionine that is given as a dietary protein supplement, as has been demonstrated in experimental nitrous oxide toxicity (7).

We have hypothesized that cisplatin-induced polyneuropathy is due to an in vivo interaction of cisplatin and B_{12} with subsequent inactivation of the cobalamin coenzymes. To investigate this hypothesis, B_{12} metabolism was studied in patients treated with cisplatin. Parameters measured included serum B_{12} and B_{12}-binding capacity, serum and urine homocystine, and urinary methylmalonic acid (MMA). Present methods of analysis precluded detection of the platinum-B_{12} complex in serum and urine.

MATERIALS AND METHODS

Thirty-one patients with advanced ovarian and bladder

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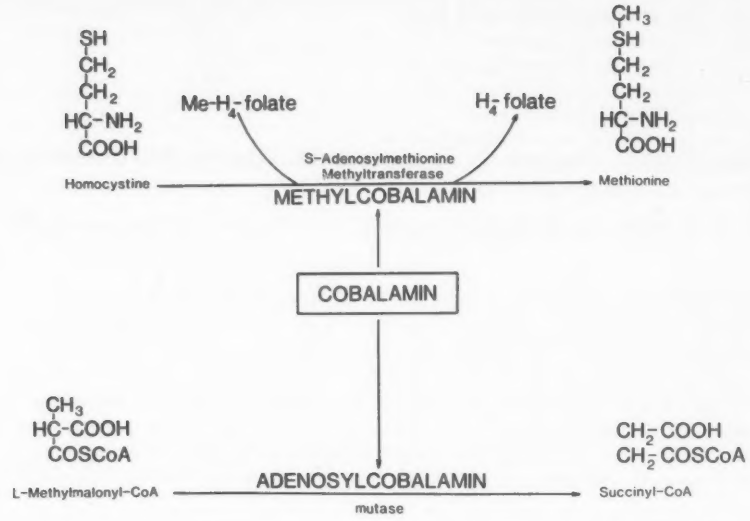

FIGURE 1.—Reactions catalyzed by cobalamin coenzymes. Cobalamin is an essential cofactor for two biochemical reactions, each of which is important for synthesis of normal myelin.

cancer were treated with nine monthly iv infusions of cisplatin (60 mg/m^2) and doxorubicin (60 mg/m^2). This was part of a protocol to assess the effect of circadian timing of drug administration on drug toxicity. Doxorubicin was given 12 hours prior to cisplatin. Serum B_{12} and B_{12}-binding capacity were measured by standard radioassay prior to chemotherapy, mid-course, and late in the course of therapy. An analysis of variance was performed upon the mean absolute values at the three timepoints. In ten patients, urinary MMA excretion was measured at 2-hour intervals during wakefulness, and once during mid-sleep during the 24 hours preceding and following cisplatin chemotherapy. These data were analyzed for circadian rhythmicity by the single cosinor method (8). MMA was determined spectrophotometrically according to the method of Giorgio and Plaut (9); t tests were used to compare values of urinary MMA excretion, expressed in millimols per hour, pre- and post-cisplatin administration. In six patients, 24-hour urinary homocystine excretion and spot plasma homocystine concentrations were measured before and after (12 and 24 hours following) cisplatin administration. A model 121M fully automated analyzer (Beckman Instruments) with a single 2.8 × 205-mm column of Beckman AA-10 ion exchange resin was used for amino acid analysis, with methods previously described (10).

RESULTS

Homocystine was undetectable in urine and plasma both before and after cisplatin administration. The assay employed was capable of determining concentrations as small as 1 mmol/100 ml. The 24-hour mean urinary MMA excretion rate was 0.03 ± 0.003 mmol both before and after cisplatin therapy. The pattern of MMA excretion prior to cisplatin therapy was found to be circadian rhythmic ($P < 0.04$). Serum B_{12} and B_{12}-binding capacity were unchanged with cisplatin therapy (table 1).

DISCUSSION

Although cisplatin binds to vitamin B_{12} in vitro at physiologic temperature and pH, this study fails to demonstrate a cisplatin-induced cellular cobalamin deficiency in treated patients. There was no abnormal accumulation of homocystine in plasma or urine, or excessive urinary excretion of MMA to suggest inadequate supply of the vitamin at the cellular level. Thus, we cannot implicate inactivation of the vitamin B_{12} coenzymes as a mechanism of cisplatin-induced neurotoxicity.

The fact that serum B_{12} levels did not change in our study suggests that cisplatin did not alter the radioimmunoassay. We have not, however, tested the effect of cisplatin on the B_{12} assay in an in vitro situation. The original observation by Hogenkamp et al (4) was that the di-aquated form of platinum formed complexes with the cobalamin coenzymes. This is presumably the intracellular, active form of cisplatin and would be expected to bind B_{12} and inactivate it within the cell. The major point of our investigation is that a cellular cobalamin deficiency was not demonstrated based upon the MMA and homocystine data.

TABLE 1.—Results of serum B_{12} and B_{12}-binding capacity during cisplatin therapy*

Time of serum sampling	Serum B_{12} ± SE (normal range, 250–900 pg/ml)	Serum B_{12}-binding capacity ± SE (normal range, 1000–2000 pg/ml)
Prior to cisplatin	422 ± 30	1391 ± 104
After 5 courses (300 mg/m²)	418 ± 44	1381 ± 110
After 9 courses (540 mg/m²)	429 ± 43	1219 ± 75

* Serum B_{12} was studied in 31 patients and B_{12}-binding capacity was studied in 24 patients. No differences were found in serum B_{12} ($n = 69$, $f = 0.02$, $P = 0.98$) or B_{12}-binding capacity ($n = 54$, $f = 0.7$, $P = 0.5$).

Since fatty acids are immediate myelin precursors, biochemical analysis of the fatty acid composition of the brain, spleen, liver, muscle, and fat tissues of rats given cisplatin weekly for 10 weeks was performed (data not shown). These studies were done in case that low levels of MMA would have been incorporated into fatty acids and tissues very efficiently, ie, with kinetics that might exclude them from reaching the urine in high concentrations. It would be expected that if the B_{12}-dependent conversion of malonic acid to malonyl CoA was impaired, MMA would be activated and incorporated into fatty acids, resulting in a relative increase in the proportion of branched to straight chain fatty acids. We found no increase in the branched chain fatty acid concentration or in the branched to straight chain fatty acid ratio in any of these five organs. Further clinical investigation of the potential utility of vitamin B_{12} treatment for cisplatin-induced neuropathy is not indicated.

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Phase II Study of Spirogermanium in Patients With Advanced Carcinoma of the Cervix[1,2]

Dean E. Brenner,[3,4,*] Neil B. Rosenshein,[5] Michael Dillon,[5] Howard W. Jones,[5] Arlene Forastiere,[4] Stuart Tipping,[4] Lonnie S. Burnett,[3] F. Anthony Greco,[3] and Peter H. Wiernik[4,6]

Spirogermanium was discovered in 1974 by Rice et al (1) in a search for azospirans with antitumor activity. The compound consists of a germanium molecule in a heterocyclic ring structure. Spirogermanium appears to inhibit protein synthesis, although DNA and RNA synthesis are not inhibited (2,3). In vitro activity was found against HeLa, L1210, human malignant lymphoma, colon carcinoma, neuroblastoma, and squamous cell carcinoma cells (2,3). Early phase I data suggested a starting dose of 50–80 mg/m^2 every other day iv for 4 weeks (4). Legha et al (5) reported maximum tolerated doses of 100 mg/m^2/day by iv bolus. Using continuous infusion over 5 days, they achieved a maximum tolerated dose of 200 mg/m^2/day. While Legha et al (5) found some activity in non-Hodgkin's lymphomas, no other solid tumor activities have been reported to date.

Seventeen patients with advanced, previously treated squamous cell carcinoma of the cervix were treated with spirogermanium at a dose of 80 mg/m^2 by iv drip over 1 hour, every other day. In 12 patients, the spirogermanium dose was escalated over the first 2 weeks of treatment to 120 mg/m^2. Characteristics of the patients are listed in table 1. These patients were all heavily pretreated. All patients had measurable disease either by pelvic examination (eight of 17) or by measurable extrapelvic disease (nine of 17). Extrapelvic disease included supraclavicular lymph nodes (six of nine patients), chest nodules (two of nine), and computed tomographic measurable para-aortic lymph nodes (one of nine). Standard criteria were used to assess response. Toxic effects were graded by Eastern Cooperative Oncology Group (ECOG) criteria.

Patients treated for a minimum of 2 weeks (six administered doses) were considered evaluable for response. Patients were treated for at least 4 weeks unless there was rapid tumor progression or severe drug toxicity. One patient was inevaluable for response due to early death (af-

TABLE 1.—Patient characteristics

	No. of patients (%)
Evaluable	17
Age (yrs)	
Median: 50	
Range: 34–62	
Karnofsky performance status	
Median: 70	
Range: 40–100	
Previous treatment	
External radiation therapy	17 (100)
Brachytherapy	15 (80)
Chemotherapy	3 (18)
Total dose administered (mg/m^2)	
Mean: 2097	
Range: 570–4554	
Survival (days)	
Mean: 123	
Range: 27–584+	
Response	0 (0)
Toxic effects*	
Lightheadedness	8 (47)
Nausea†	5 (29)
Lethargy	5 (29)
Phlebitis	5 (29)
Fatigue	4 (24)
Vomiting†	3 (18)
Blurred vision	3 (18)
Metallic taste	3 (18)
Dyspnea	1 (6)
Dry mouth	1 (6)

* All toxic effects, ECOG grade 1 or 2.
†1 patient, ECOG grade 3.

[1]Received June 21, 1984; revised Oct 18, 1984; accepted Oct 25, 1984.
[2]Unimed, Inc supplied the spirogermanium for clinical use.
[3]Division of Oncology, Department of Medicine (*D. E. Brenner* and *F. A. Greco*), and Division of Gynecologic Oncology, Department of Obstetrics and Gynecology (*H. W. Jones* and *L. S. Burnett*), Vanderbilt University, Nashville, TN.
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[6]We thank Mrs. Alice Hammond for assistance with preparation of the manuscript.
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ter three doses). Her death was felt to be due to advanced tumor, not drug toxicity. No patient responded to treatment.

As in previous studies, the drug was well-tolerated. No myelosuppression was observed. Dose-limiting toxic effects of nausea and vomiting occurred in one patient at 80 mg/m^2. The patient was azotemic (creatinine = 5.7 mg/100 ml), and the toxicity was thought to be due to a combination of azotemia and the drug. Her nausea and vomiting improved following discontinuation of the drug.

Our experience leads us to conclude that spirogermanium administered on an escalating every other day schedule to a maximum of 120 mg/m^2 is inactive (< 20% response rate, with 95% confidence) in patients with previously treated, advanced squamous cell carcinoma of the cervix. This is unfortunate, because spirogermanium was well-tolerated and had nonoverlapping toxic effects with other cytotoxic agents, properties which make it an ideal agent for inclusion in multiagent regimens. It is possible that escalation to higher doses (> 120 mg/m^2) by infusion may induce tumor responses.

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Phase II Trial of 1,2-Diaminocyclohexane-(4-carboxyphthalato) Platinum(II) in Patients With Refractory Germ Cell Tumors[1]

Hoo Chun, George J. Bosl,* and Robert B. Golbey[2]

1,2-Diaminocyclohexane-(4-carboxyphthalato) platinum(II) (DACCP) is a second generation platinum analog that was selected for clinical trial because of a lack of in vitro cross-resistance with cisplatin-resistant P388 and L1210 cell lines (1), decreased nephrotoxicity in preclinical screening systems, and enhanced solubility relative to other platinum analogs (2,3). A phase I trial conducted at Memorial Sloan-Kettering Cancer Center recommended a starting dose of 640 mg/m^2 (4). The present study was designed to evaluate the effectiveness of DACCP in the treatment of patients with germ cell tumors (GCT) refractory to conventional therapy.

All patients entered in this study had histologically confirmed GCT. All patients had failed to achieve a complete remission with both standard induction chemotherapy regimens using high doses of cisplatin ($\geq$ 100 mg/m^2), vinblastine, and bleomycin (with or without cyclophosphamide and dactinomycin), and "salvage" chemotherapy regimens with etoposide plus cisplatin. All patients had progressive disease at the time of treatment with DACCP. The extent of disease was determined by physical examination, chest x-ray, computerized tomography when indicated, screening biochemistry profile, and serum tumor markers, including lactate dehydrogenase (LDH), alpha-fetoprotein (AFP), and human chorionic gonadotropin (HCG). All patients had one or more bidimensionally measurable lesions, Karnofsky performance status $\geq$ 50%, life expectancy of at least 6 weeks, wbc count $\geq$ 3000/mm^3, platelet count $\geq$ 100,000/mm^3, serum bilirubin < 2.0 mg/dl, BUN $\leq$ 30 mg/dl, serum creatinine $\leq$ 1.5 mg/dl, or creatinine clearance $\geq$ 50 ml/minute. All prior therapy had been discontinued at least 4 weeks before entry into this study. Written informed consent was obtained from all patients. DACCP was supplied by the Johnson-Matthey Corporation as a lyophilized powder and was prepared immediately prior to infusion over 15–20 minutes. The starting dose was 640 mg/m^2 every 3 weeks. Hydration consisted of 500 ml of 5% dextrose in 0.45% NaCl given iv

TABLE 1.—Characteristics of patients treated with DACCP

Characteristics	No. of patients
Entered	9
Evaluable	9
Median age in yrs (range): 23 (17–53)	
Median Karnofsky performance status (range): 80 (50–90)	
Primary site	
Testis	7
Retroperitoneum	2
Prior chemotherapy*	
VAB-6	6
PVB	4
Etoposide + cisplatin	9
Other phase II	2
Median prior cycles of cisplatin $\geq$ 100 mg/m^2 (range): 7 (5–9)	
Sites of metastasis	
Lung	6
Retroperitoneum	5
Liver	2
Supraclavicular lymph node	1
Markers elevated	
LDH	8
AFP	6
HCG	5
Responses†	0
Toxicity	
Nausea and vomiting	6
Diarrhea	3
Fever	2
Median wbc count nadir (range) 5400/mm^3 (3900–10,500)	
Median platelet count nadir (range) 163,000/mm^3 (42,000–407,000)	

*Evaluable patients only. VAB-6 = vinblastine, bleomycin, cisplatin, dactinomycin, and cyclophosphamide; PVB = cisplatin, vinblastine, and bleomycin.

†Complete + partial responses.

[1]Received June 29, 1984; revised Oct 5, 1984; accepted Oct 30, 1984.

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before and after therapy. In the absence of demonstrable toxicity, subsequent doses could be escalated by 80 mg/m^2. The maximum allowable dose was 800 mg/m^2. Response was defined as previously described (5).

All nine male patients entered into this trial were evaluable (table 1). No responses were seen (true predicted response rate $\leq$ 30%; $P < 0.05$). Dosage escalation to 720 mg/m^2 was accomplished in four patients and to 800 mg/m^2 in one. Toxicity was mild (table 1). A change in renal function (two patients) and a decrease in platelet count (six) were observed as evidence of the drug's biologic activity. In the ninth patient, the HCG value decreased from 1000 to 141 ng/ml and the LDH from 3340 to 230 units/L.

DACCP, given in the dose and schedule in this trial, had no therapeutic activity in patients with GCT refractory to conventional chemotherapy. Unfortunately, the trial closed early due to an inability to obtain further drug. However, the absence of major objective responses suggests that DACCP shares considerable cross-resistance with cisplatin. The testing of new active agents, including other platinum analogs in GCT, should be continued.

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Phase II Trial of Mitoguazone in Patients With Refractory Germ Cell Tumors[1,2]

Hoo Chun and George J. Bosl[3,*]

Mitoguazone (methyl-GAG) is one of several synthetic polycarbonyl compounds whose antiproliferative activity against L1210 leukemia was first reported in 1958 (1). Its anticancer activity may result from an inhibition of *S*-adenosylmethionine decarboxylase, an important enzyme in the biosynthesis of polyamines. Early clinical trials of methyl-GAG given on a daily schedule were associated with prohibitive toxic effects, although activity was seen in hematologic malignancies and some solid tumors. Recently, methyl-GAG was re-evaluated using a less toxic weekly iv dose schedule, and responses were observed in malignant lymphoma, head and neck cancer, esophageal cancer, and lung cancer (1,2). Because of its relative lack of myelosuppression, the efficacy of methyl-GAG was evaluated in a phase II trial of patients with advanced germ cell tumors (GCT) refractory to conventional therapy.

All patients entered in this study had histologically confirmed GCT. All patients except one had failed to achieve a complete remission with both standard induction chemotherapy regimens, using high doses of cisplatin ($\geq$ 100 mg/m^2) such as VAB-6 (vinblastine, bleomycin, cisplatin, dactinomycin, and cyclophosphamide) (3) or PVB (cisplatin, vinblastine, and bleomycin) (4), and "salvage" chemotherapy regimens with etoposide plus cisplatin. The one exception was a patient who had achieved a brief complete remission of 6 months' duration. All patients had progressive disease at the time of treatment with methyl-GAG. The extent of disease was determined by physical examination, chest x-ray, computerized tomography when indicated, screening biochemistry profile, and serum tumor markers, including lactic dehydrogenase, alpha-fetoprotein, and human chorionic gonadotropin. All patients had one or more bidimensionally measurable lesions, Karnofsky performance status $\geq$ 50%, life expectancy of at least 6 weeks, wbc count $\geq$ 3000/mm^3, platelet count $\geq$ 100,000/mm^3, serum bilirubin < 1.5 mg/dl, fasting blood sugar $\geq$ 70 mg/dl, and creatinine clearance $\geq$ 50 ml/minute. All prior therapy

TABLE 1.—Characteristics of patients treated with methyl-GAG

Characteristics	No. of patients
Entered	16
Evaluable	14
Primary site	
Testis	14
Mediastinum	1
Retroperitoneum	1
Prior chemotherapy*	
VAB-6 (ref 3)	11
PVB (ref 4)	3
Etoposide + cisplatin	14
Other phase II	2
Median prior cycles of cisplatin $\geq$ 100 mg/m^2 (range): 6 (4-9)	
Sites of metastasis	
Lung	9
Retroperitoneum	9
Liver	4
Supraclavicular lymph node	2
Chest wall	1
Peritoneum	1
Markers elevated†	
LDH	16/16
AFP	6/16
HCG	10/16
Responses‡	0/14
Toxicity	
Nausea	7
Vomiting	5
Mucositis	5
Muscle weakness	5
Facial flushing	2
Diarrhea	1
Median wbc count nadir (range) 6200/mm^3 (2900-10,000)	
Median platelet count nadir (range) 171,000/mm^3 (54,000-286,000)	

* Evaluable patients only.

†LDH = lactate dehydrogenase; AFP = alpha-fetoprotein; HCG = human chorionic gonadotropin.

‡Complete + partial resonses.

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[3]Solid Tumor Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, and Department of Medicine, Cornell University Medical College, New York, NY.

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had been discontinued for at least 4 weeks before entry in this study. Written informed consent was obtained from all patients. The drug was administered through the side arm of a running iv line over 45–60 minutes at a starting dose of 600 mg/m^2 weekly for the first two to four doses and then every other week thereafter. Response was defined as previously described (5).

Sixteen male patients entered this trial (table 1). Two patients received only one dose and are considered inevaluable. One patient developed a complete epidural block 5 days after the initial dose, and the second was found to be a protocol violation with an inadequate initial platelet count. Neither patient had any evidence of response. No responses were observed in 14 evaluable patients (predicted true response rate $\leq 20\%$; $P < 0.05$). Six patients developed rapidly progressive disease after only two doses, necessitating a change in treatment. Eight patients received three or more doses. Toxicity was minimal (table 1); no nephrotoxicity was observed.

Methyl-GAG was inactive as a single agent in patients with refractory GCT at this dose and schedule. All patients rapidly progressed during therapy. Given the lack of myelosuppression, the absence of efficacy was disappointing. However, the evaluation of new agents in GCT must continue in order that new treatment strategies can be designed for those patients most likely to die of their disease.

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Exploring the Future of Cancer Care

Pasadena, California, April 24, 1985

The University of Southern California is the sponsor of this meeting, which will be held at the Pasadena Hilton Hotel. The fee (luncheon included) is $60.00 ($25.00 for Cancer Management Network full-member hospitals).

For further information, contact: Linda Richie-Walker, Cancer Management Network, 1721 Griffin Ave, Phinney Hall, Rm 205, Los Angeles, CA 90031 (telephone: 213-224-7368).

Second Annual Lykes Cancer Center Symposium

Clearwater Beach, Florida, May 17–18, 1985

The theme of this multidisciplinary conference is head and neck cancer. The meetings, which will be held at the Holiday Inn Surfside, should be of interest to physicians, dentists, nurses, pharmacists, dental hygienists, therapists, and rehabilitative professionals; 12 hours of Continuing Education credits are approved.

For further information, contact: Clearwater Seminars, P.O. Box 1027, Clearwater, FL 33517 (telephone: 813-443-1081).

Seventy-Sixth Annual Meeting of the American Association for Cancer Research

Houston, Texas, May 22–25, 1985

The program of this conference will consist of slide and poster sessions, minisymposia, poster discussion sessions, special lectures, and the AACR's annual business meeting. In addition, there will be symposia on recent progress in cancer research, mechanisms of tumor progression, cellular mechanisms of drug resistance, and oncogenes. The symposium on oncogenes will be sponsored jointly with the American Society of Clinical Oncology, which will hold its 21st annual meeting, also at the Houston Civic Center, on May 19–21, 1985.

Registration for the AACR meeting will take place in the East Hall of the Albert Thomas Center beginning at noon on May 21 and will continue throughout the meeting.

For further information, contact: American Association for Cancer Research, West Bldg, Rm 301, Temple University School of Medicine, Philadelphia, PA 19140 (telephone: 215-221-4565).

Breast Preservation: Workshop on the Technique of Conservative Surgery and Radiotherapy for Early Breast Cancer

New York, New York, June 14–15, 1985

The Continuing Medical Education Office of Memorial Sloan-Kettering Cancer Center is sponsoring this postgraduate workshop with an emphasis on defining, explaining, and providing a rationale for the techniques currently in use for the treatment of breast cancer. Twenty-six invited faculty from the United States and Europe will interact based on their experience and that of their institutions. The program has been approved for 9 hours of CME credit, Category I.

For further information, contact: CME Conference Planning Office, C-180, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021 (telephone: 212-794-6754).

International Symposium on Labelled and Unlabelled Antibody in Cancer Diagnosis and Therapy

Baltimore, Maryland, September 12–13, 1985

This symposium, to be held at The Johns Hopkins Medical Institutions, will include sessions on monoclonal antibody in therapy, treatment of bone marrow with antibody for transplantation, radiolabeled antibody in diagnosis, dosimetry and physics of radiolabeled antibody, radiobiology of low-dose-rate radiation, therapeutic approaches with drug labels, radiolabeled antibody, and new diagnostic and therapeutic approaches.

The deadline for submission of abstracts is *May 1, 1985*.

For further information, contact: American College of Radiology, 925 Chestnut St, Philadelphia, PA 19107 (telephone: 215-574-3150).

SOMPS First International Congress on Neo-Adjuvant Chemotherapy

Paris, France, November 7–9, 1985

Several international experts will participate in this congress, sponsored by the Service d'Oncologie Médicale Pitié-Salpétriere (SOMPS). Abstracts should be submitted to the organizing committee by *July 1, 1985*.

For further information, contact: SOMPS—First International Congress on Neo-Adjuvant Chemotherapy, Pavillon Jacquart, Hôpital de la Salpétrière, 47, boulevard de l'Hôpital, 75651 Paris Cedex 13, France (telephone: 570-28-29 or 570-28-30).

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